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ASSIMILATION OF IRON BY RICE FROM CERTAIN NUTRIENT SOLUTIONS

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INTRODUCTION

It has long been recognized that on calcareous soils certain plants do not make a normal growth and are often affected by chlorosis. This has been variously ascribed to the physical condition of the soil; an increasing assimilation of lime by the plant; a diminished assimilation of potash, iron, or phosphoric acid; or a diminished assimilation of all mineral nutrients due to the neutralization of acid root excretions. As already noted, work at this station with pineapples and upland rice indicated an insufficient assimilation of iron as the principal cause (4, 5)[1]

As calcareous soils have a slightly alkaline reaction it is important to know whether plants intolerant of calcareous soils are sensitive to an acid or alkaline reaction *per se*, and whether the reaction of the soil has any effect on the assimilation of iron. Various experiments with upland rice (*Oryza sativa*) in nutrient solutions and soil cultures have been conducted to gain information on this subject. The experiments with nutrient solutions reported here show the effect of the quantity and form of iron and reaction of the nutrient medium on the asssimilation of iron by rice.

PLAN AND EXPERIMENTAL METHODS

The plan of the work was to measure the growth of rice in acid, neutral, and alkaline nutrient solutions when supplied with 0.002 and 0.008 gm. of iron (Fe) per liter from ferrous sulphate, ferric chlorid, dialyzed iron, ferric citrate, and ferric tartrate; to determine the amount of iron taken up by the plants from these solutions; and to determine the amount of soluble iron actually present in the solutions at different times. A few other experiments were conducted to explain certain results obtained. Three preliminary experiments were also conducted before the general plan and exact detail of the experiments were decided upon.

[1] Reference is made by number to "Literature cited," p. 528.

Upland rice seedlings germinated over distilled water were placed in the nutrient solutions when the plumules were about 2 inches long. Water distilled from a cast-iron still with a block-tin condenser and stored in a tin-lined copper tank was used in the nutrient solutions. The plants were grown for 40 days in Erlenmeyer flasks of Jena or Nonsol glass. Flasks of 200 c. c. capacity were used for the first 25 days of each experiment and flasks of 500 c. c. capacity for the remaining 15 days. Transpired water was replaced with distilled water daily, and the plants were changed to fresh solutions every 4 days, except they were left in the solution 6 days before the first change. The plants were kept in a wire inclosure (five meshes to the inch) during fair weather and in a glass house during rains.

The nutrient solutions used were of the following compositions:

Acid solution.	*Gm.*	Neutral solution.	*Gm.*
Potassium nitrate (KNO_3)	10. 71	Potassium nitrate (KNO_3)	10. 71
Monobasic potassium phosphate (KH_2PO_4)...	7. 14	Monobasic potassium phosphate (KH_2PO_4) ..	3. 57
Sodium nitrate ($NaNO_3$)..	21. 43	Dibasic potassium phosphate (K_2HPO_4).....	3. 57
Sodium sulphate (Na_2SO_4)	3. 15	Sodium nitrate ($NaNO_3$)..	21. 43
Calcium chlorid ($CaCl_2$)..	2. 0	Sodium sulphate (Na_2SO_4)	3. 15
Magnesium chlorid ($MgCl_2$).....	2. 0	Calcium chlorid ($CaCl_2$)..	2. 0
Sulphuric acid (H_2SO_4)...	0. 245	Magnesium chlorid ($MgCl_2$).....	2. 0
Distilled water.....	100, 000	Distilled water.....	100, 000

The solution alkaline with carbonate of lime was the same as the neutral solution except for the addition of 0.41 gm. of precipitated calcium carbonate per liter. In the various experiments different quantities and kinds of iron were added to these solutions.

It is possible that the above solutions are not ideal for rice, although plants equal in size to exceptionally large field plants were grown in them. Previous and subsequent work with these solutions showed that the growth of rice was not increased by increasing the quantity of calcium chlorid or magnesium chlorid, by doubling the quantities of dibasic potassium phosphate and monobasic potassium phosphate, or by doubling the quantities of potassium nitrate and sodium nitrate. The concentration of total salts in the solutions was lower than usual, but afforded sufficient nutrients because of the frequent changes. There is an advantage in using dilute solutions in that imperfect balancing of the salts (antagonistic effects of the ions) may produce no injury in low concentrations.

Before each change the nutrient solutions were made up fresh from a stock solution and the iron added 18 hours before the plants were inserted. After emptying out the old solution, the flasks and roots were rinsed once with a small quantity of distilled water. These details seem unimportant, but, as will be seen later, alteration in such apparently trivial details may affect the results.

In analyzing the plants for iron the substance was ashed over a low flame without the addition of calcium acetate and iron determined colorimetrically by the method of Stokes and Cain (14). In attempting to estimate the soluble iron in the nutrient solutions, the solutions were filtered, the filtrate concentrated when necessary, and iron determined colorimetrically by the above method. The ordinary colorimetric method with potassium sulphocyanate (KSCN) was used in some of the preliminary work, but all the determinations reported were made by the method of Stokes and Cain unless otherwise specified. Blanks were run for iron with the acid and materials employed.

PRELIMINARY EXPERIMENTS

Following are the data of three preliminary experiments conducted before a uniform method was adopted:

EXPERIMENT 1 (Source of iron: Ferrous sulphate. Plants grown in double flasks).—It was at first thought it would be advantageous to grow the plants with their roots equally divided between two flasks, one flask to contain the acid solution and the other the neutral. By adding iron only to the acid solution in one lot and only to the neutral solution in another lot it was thought that decisive results would be obtained on the effect of the reaction of the solution on the assimilation of iron, as the only difference between the two lots would be in the solution in which iron occurred. The results, however, did not bear out the assumption.

Two plants were grown in each twin flask (A and B). Four flasks were taken as a unit and the units triplicated for each treatment. All solutions containing iron contained the same quantity, but in this experiment the quantity of iron was not kept constant during the 40 days of growth, as it was thought that at first so much iron was added as to obscure the effect of the reaction on assimilation. During the first 10 days 0.008 gm. of iron per liter was used, during the second 10 days 0.004 gm. of iron, and during the last 20 days 0.002 gm. of iron. In Table I are given the weights of the plants grown in the different solutions, together with the percentages of nitrogen and iron in the dry substance.

The color of the plants during growth was as follows: At 10 days plants 1 to 12 were strongly chlorotic, plants 25 to 36 were chlorotic, plants 13 to 24 and 49 to 60 were green, and plants 37 to 48 were dark green; from the twentieth to the fortieth day plants 1 to 12 were strongly chlorotic and all others were about the same normal green.

From the weights of the stalks and leaves it appears that with the quantities of iron used the reaction of the solution had little effect on the assimilation of iron. It also seems that there is no advantage in growing the plants with their roots divided between two flasks, as one-half the roots are not able to absorb sufficient iron for the full needs of

the plants.[1] This is evident from a comparison of the weights of plants 13 to 24, with 37 to 48, and of plants 25 to 36 with 49 to 60.

TABLE I.—*Comparative weights of rice plants grown in double flasks with ferrous sulphate in acid and neutral solutions and percentage of nitrogen and iron*

Nutrient solution in flask—		Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of stalks and leaves.	Average oven-dry weight of whole plant.	Iron (Fe_2O_3) in dry stalks and leaves.	Nitrogen (N) in dry stalks and leaves.
A.	B.								
			Gm.	*Gm*	*Gm.*	*Gm.*	*Gm.*	*Per ct.*	*Per ct.*
Neutral	Acid	1–4	1. 42	0. 26	0. 10	.....	.....	.....	.....
		5–8	1. 39	. 26	. 09	.....	.....	.....	.....
		9–12	1. 32	. 26	. 10	0. 26	0. 36	0. 040	3. 90
Do	Acid+iron	13–16	36. 10	4. 78	1. 29	.....	.....	.....	.....
		17–20	38. 55	5. 09	1. 39	.....	.....	.....	.....
		21–24	28. 85	3. 93	1. 10	4. 60	5. 86	. 038	3. 72
Neutral+iron.	Acid	25–28	29. 62	4. 10	1. 14	.....	.....	.....	.....
		29–32	33. 80	4. 78	1. 23	.....	.....	.....	.....
		33–36	31. 19	4. 27	1. 17	4. 38	5. 56	. 023	3. 90
Acid+iron	Acid+iron	37–40	48. 42	5. 99	1. 87	.....	.....	.....	.....
		41–44	48. 70	6. 22	1. 88	.....	.....	.....	.....
		45–48	51. 97	6. 61	1. 91	6. 27	8. 16	. 038	3. 70
Neutral+iron.	Neutral+iron.	49–52	49. 25	6. 34	1. 67	.....	.....	.....	.....
		53–56	53. 67	7. 00	1. 91	.....	.....	.....	.....
		57–60	50. 49	6. 48	1. 70	6. 61	8.37	. 022	3. 80

EXPERIMENT 2 (Source of iron: Ferrous sulphate. Three different quantities of iron in acid and neutral solutions).—Since it was thought that in experiment 1 so much iron was used as to obscure partially the effect of the reaction on the assimilation of iron, an experiment was conducted using different quantities of iron in acid and neutral solutions. As a check on the previous results, two lots received iron at the same rate as in experiment 1—that is, 0.008 gm. of iron per liter for the first 10 days, 0.004 gm. for the second 10 days, and 0.002 gm. for the last 20 days of the experiment. The other lots received either 0.008 or 0.002 gm. per liter during the whole 40 days. Ferrous sulphate was used as the source of iron. One plant was grown in each flask, six flasks were taken as a unit, and the units triplicated for each treatment. The weights of the plants with the percentages of nitrogen, phosphoric acid, and iron in the dry substance are given in Table II.

The color of the plants during growth was as follows: After 7 days' growth plants 55 to 72 were yellowish green, plants 91 to 108 and 73 to 90 were light green, plants 1 to 54 were dark green; after 12 days' growth plants 55 to 72 were light green and all others were of a good green color.

It is apparent from this experiment that the effect of the reaction of the solution depends somewhat on the quantity of iron supplied, this reaction being more evident in solutions containing a small amount of iron.

[1] This is not definitely proved by this experiment alone, but further work substantiated it.

TABLE II.—*Comparative weights of rice plants grown in acid and neutral solutions with three quantities of iron from ferrous sulphate*

Nutrient solution.	Quantity of iron per liter.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—		In dry substances of stalks and leaves, percentages of—		
						Stalks and leaves.	Whole plant.	Nitrogen (N)	Phosphoric acid (P_2O_5).	Iron (Fe_2O_3).
	Gm.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*P. c.*	*P. c.*	*P. c.*
Acid	0.002	1– 6	32.05	4.04	1.04	....	....	....	....	.....
		7–12	32.98	4.12	1.08	....	....	....	....	.....
		13– 18	31.40	3.93	.92	4.03	5.04	4.34	2.31	0.037
Do	.008, 0.004, 0.002	19– 24	30.48	3.90	.96	....	....	....	....	.....
		25– 30	30.00	3.78	.95	....	....	....	....	.....
		31– 36	34.38	4.18	.91	3.95	4.89	4.55	2.20	.037
Do	.008	37– 42	34.72	4.37	1.32	....	....	....	....	.....
		43– 48	53.10	6.58	1.92	....	....	....	....	.....
		49– 54	51.18	6.12	1.80	5.69	7.37	4.40	2.02	.034
Neutral	.002	55– 60	28.48	3.73	.85	....	....	....	....	.....
		61– 66	26.71	3.34	.79	....	....	....	....	.....
		67– 72	27.72	3.56	.79	3.54	4.35	4.56	2.06	.024
Do	.008, 0.004, 0.002	73– 78	36.42	4.40	1.03	....	....	....	....	.....
		79– 84	37.75	4.69	1.15	....	....	....	....	.....
		85– 90	28.28	3.62	.84	4.24	5.24	4.40	2.04	.020
Do	.008	91– 96	59.18	7.54	2.17	....	....	....	....	.....
		97–102	....	....	....	....	....	....	....	.....
		103–108	59.45	7.49	2.00	7.52	9.60	4.28	1.80	.028

EXPERIMENT 3 (Sources of iron in acid, neutral, and alkaline solutions: Ferrous sulphate and ferric citrate).—This experiment was designed to observe the assimilation of iron in a solution containing calcium carbonate—that is, a slightly alkaline solution—and to compare the assimilability of ferric citrate with that of ferrous sulphate. The method used in the conduct of this experiment differed from that of other experiments only in the making of the nutrient solutions. In all other experiments reported the nutrient solutions were made up fresh 18 hours before the plants were inserted, and any residue remaining in the bottles from the previous lot was thrown away. In this experiment the nutrient solutions were made up fresh 18 hours beforehand, but whatever nutrient solution remained from the previous change was left in the bottles and added to the fresh solutions. This residue increased at times but never amounted to more than a quarter of the whole solution. It nevertheless affected the results.

Iron from both ferric citrate and ferrous sulphate was used at the rate of 0.002 gm. of iron per liter in all the cultures. The alkaline solutions with calcium carbonate contained 0.41 gm. of precipitated calcium carbonate per liter. One plant was grown in each flash. Six flasks were taken as a unit, and the units were triplicated for each treatment. The growths of the plants in the different solutions and a partial ash analysis of the stalks and leaves are given in Table III.

TABLE III.—*Comparative weights of rice plants grown in acid, neutral, and alkaline solutions with 0.002 gm. of iron per liter from ferrous sulphate or ferric citrate*

Nutrient solution.	Source of iron.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—		Composition of stalks and leaves.					
						Stalks and leaves.	Whole plant.	Carbon-free ash.	Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Phosphoric acid (P_2O_5).	Iron (Fe_2O_3).
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Acid	Ferrous sulphate	1–6	38.44	4.57	1.12								
		7–12	37.32	4.49	1.07								
		13–18	32.60	4.05	1.03	4.37	5.44	13.90	0.26	0.74	1.18	2.03	0.044
Do	Ferric citrate	19–24	46.02	5.13	1.21								
		25–30	40.24	4.63	1.14								
		31–36	43.64	5.13	1.26	4.96	6.16	14.41	.13	.75	1.18	1.97	.039
Neutral	Ferrous sulphate	37–42	25.25	2.99	.74								
		43–48	29.67	3.48	.83								
		49–54	26.66	3.20	.76	3.22	3.99	14.19	.28	.79	1.44	2.03	.028
Do	Ferric citrate	55–60	38.14	4.45	1.06								
		61–66	31.78	3.79	.92								
		67–72	38.78	4.45	1.02	4.23	5.23	14.23	.22	.78	1.15	1.94	.024
Alkaline	Ferrous sulphate	73–78	13.20	1.76	.70								
		79–84	21.05	2.60	1.07								
		85–90	19.15	2.34	.85	2.23	3.10	13.64	.39	.96	1.23	2.17	.026
Do	Ferric citrate	91–96	33.30	3.98	1.25								
		97–102	39.72	4.78	1.51								
		103–108	33.64	4.09	1.43	4.28	5.68	14.22	.22	.87	1.16	2.02	.021

The color of the plants during growth was as follows: After 8 days growth plants 1 to 18 were a good green, and all others were strongly chlorotic; after 15 days growth plants 1 to 36 were a good green, 91 to 108 were slightly chlorotic, 55 to 72 were chlorotic, 73 to 90 and 37 to 54 were strongly chlorotic; after 23 days growth plants 1 to 36 were dark green, 91 to 108 and 55 to 72 were light green, 37 to 54 were a lighter green, and 73 to 90 were chlorotic.

In this experiment there was a greater difference in growth between the acid and neutral solutions with 0.002 gm. of iron per liter from ferrous sulphate than in the preceding experiment, probably due to the different method of preparing the solutions. The residual solutions not only contained less soluble iron than the freshly prepared solutions, but the precipitate already formed in the residual solutions probably affected the rate at which the iron in the freshly prepared solutions became insoluble.

RESULTS OF PRELIMINARY EXPERIMENTS

The three preliminary experiments showed that the effect of the reaction of the solution on growth and assimilation of iron depended somewhat on the quantity of iron added. With 0.002 gm. of iron per liter from ferrous sulphate, growth was greatest in the acid solution, but with 0.008 gm. of iron per liter, growth was greatest in the neutral solution.

Increasing the quantity of iron from 0.002 to 0.008 gm. per liter greatly increased the growth in both the acid and neutral solutions.

Analyses showed that plants grown in the different solutions did not differ appreciably in their percentages of nitrogen, phosphoric acid, lime, magnesia, or carbon-free ash, but did differ materially in the quantity of iron they contained. Plants grown in the acid solutions contained about 50 per cent more iron than plants grown in corresponding neutral or alkaline solutions.

In the course of this and other work it has been found that the color of the leaves is a pretty good indication of whether or not the plant is obtaining sufficient iron, although a lack of green is often attributable to other causes than a lack of iron. During growth, plants in the acid solution were the darkest green, in accordance with the percentages of iron found in the plants.

Used at the rate of 0.002 gm. of iron per liter ferric citrate was plainly a better source of iron than ferrous sulphate, especially in neutral and alkaline solutions.

That the growth in the different solutions was largely controlled by the supply of available iron seems proved by the color and analyses of the plants, and by the increased growth following an increase in quantity or change in the kind of iron added to the solutions.

FINAL EXPERIMENTS

EXPERIMENT 4 (Sources of iron in acid, neutral, and alkaline solutions: Ferrous sulphate and ferric citrate).—In this experiment 0.002 and 0.008 gm. of iron per liter from ferrous sulphate and 0.002 gm. of iron from ferric citrate were tested in the three different nutrient solutions. Two plants were grown in each flask and six flasks were taken as a unit, the units being duplicated for each treatment. The data of growth and the percentages of iron in the dry substance of the stalks and leaves are given in Table IV.

The color of the plants during growth was as follows: At 10 days plants 1 to 24 and 49 to 60 were a good green, all others were a yellowish green except plants 85 to 96, which were strongly chlorotic; at 20 days plants 1 to 24 and 49 to 72 were a good green, plants 25 to 48, 97 to 108, 73 to 84, and 97 to 108 were a slightly poorer color, and plants 85 to 96 were almost white.

The results of this experiment confirm those of the preliminary experiments in respect to (1) the superiority of ferric citrate to ferrous sulphate as a source of iron, (2) the relative growths in acid and neutral solutions with the two quantities of iron, and (3) the markedly higher percentages of iron in plants grown in the acid solutions. The new fact established was that increasing the iron from 0.002 to 0.008 gm. per liter in the alkaline solution depressed the growth of plants to a surprising degree. That the lack of growth in this solution was due to a lack of available iron is

proved by the pronounced chlorosis of the plants and by the results of experiment 10. Here, as well as in experiment 1, plants 85 to 96, which made practically no growth because of a lack of iron, contained a high percentage of iron in the dry substance. This point will be discussed later.

TABLE IV.—*Comparative weights of plants grown in acid, neutral, and alkaline solutions, with ferrous sulphate or ferric citrate as the source of iron*

Nutrient solution.	Source of iron.	Quantity of iron per liter.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—		Iron (Fe_2O_3) in dry stalks and leaves.
							Stalks and leaves.	Whole plant.	
		Gm.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per ct.*
Acid	Ferrous sulphate	0.002	1–6	48.45	6.66	1.47	.....	.....	.....
Do	.....do	.002	7–12	45.85	6.42	1.46	6.54	8.01	.036
Do	.....do	.008	[a] 13–18	91.88	12.41	3.10	.....	.....	.....
Do	.....do	.008	19–24	83.29	10.94	2.65	11.68	14.56	.032
Do	Ferric citrate	.002	25–30	65.47	8.70	1.82	.....	.....	.....
Do	.....do	.002	31–36	60.07	8.47	1.83	8.59	10.42	.026
Neutral	Ferrous sulphate	.002	37–42	47.19	6.51	1.45	.....	.....	.....
Do	.....do	.002	43–48	42.39	5.92	1.35	6.21	7.61	.017
Do	.....do	.008	49–54	100.22	12.93	2.97	.....	.....	.....
Do	.....do	.008	55–60	100.31	13.05	2.90	12.99	15.93	.023
Do	Ferric citrate	.002	61–66	62.07	8.32	1.83	.....	.....	.....
Do	.....do	.002	67–72	58.60	7.85	1.79	8.09	9.90	.015
Alkaline	Ferrous sulphate	.002	73–78	45.18	6.17	1.63	.....	.....	.....
Do	.....do	.002	79–84	42.13	6.22	1.68	6.20	7.86	.017
Do	.....do	.008	85–90	.93	.21	.11	.....	.....	.....
Do	.....do	.008	91–96	1.07	.23	.11	.22	.33	.049
Do	Ferric citrate	.002	97–102	63.28	8.33	2.20	.....	.....	.....
Do	.....do	.002	103–108	61.85	8.29	2.11	8.31	10.47	.012

[a] Results calculated from five flasks.

EXPERIMENT 5 (Ferrous sulphate in neutral solution with and without carbon black).—Data of growth in the different solutions seemed to point overwhelmingly to the iron supply as the factor controlling growth; nevertheless, it was possible that in some cases the precipitate in the solutions affected growth aside from any influence of the precipitate on the amount of soluble iron. After the addition of iron salts to the nutrient solutions a precipitate was formed which varied according to the source of the iron and the nutrient solution, but was greater as the amount of iron added became larger. In the preceding experiments a marked increase in growth was produced by increasing the iron from 0.002 to 0.008 gm. per liter in the neutral solution. It was possible that the greater precipitate in the solution with 0.008 gm. of iron favored growth in removing by absorption traces of heavy metals or other substances present in the distilled water. As carbon black and ferric hydroxid have been found to improve the quality of distilled water in this way, the following test was conducted.

To two lots of solutions carbon black was added at the rate of 0.0432 gm. per liter. This is about double the weight of the precipitate in the ordinary neutral solution with 0.008 gm. of iron per liter from ferrous sulphate. Two other solutions were made up using distilled water that had previously been treated with 0.086 gm. of carbon black per liter and then filtered. The neutral nutrient solution was used in all sets, and ferrous sulphate was the source of the iron. Two plants were grown in each flask, six flasks were taken as a unit, and the units duplicated for each treatment. The results of the test are given in Table V.

TABLE V.—*Comparative weights of rice plants grown in neutral nutrient solution with iron from ferrous sulphate and also with distilled water treated with carbon black*

Kind of distilled water used in nutrient solution.	Quantity of iron per liter.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—	
						Stalks and leaves.	Whole plant.
	Gm.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Ordinary distilled water	+0.002	1–6	62.19	8.27	1.86	.....	.....
		7–12	65.07	8.48	1.92	8.38	10.27
Do.	+.008	13–18	128.62	16.31	3.73	.....	.....
		19–24	137.50	16.73	4.01	16.52	20.39
Ordinary distilled water + carbon black.	+.002	25–30	58.56	7.65	1.69	.....	.....
		31–36	57.01	7.55	1.71	7.60	9.30
Do.	+.008	37–42	139.40	17.54	3.54	.....	.....
		43–48	127.70	15.91	3.47	16.73	20.24
Do.	+.002	49–54	88.20	11.10	2.56	.....	.....
		55–60	88.10	10.97	2.49	11.04	13.57
Do.	+.008	61–66	148.53	18.49	4.42	.....	.....
		67–72	147.87	18.62	4.65	18.56	23.10

During growth lots with 0.002 gm. of iron per liter were of a slightly poorer color than lots with 0.008 gm., but no differences in color were apparent between the lots with different distilled waters or with carbon black.

It is evident that the addition of carbon black to the nutrient solutions with 0.002 gm. of iron slightly depressed rather than increased the yield while carbon in the solution with 0.008 gm. of iron had no effect on the yield. Treating the distilled water with carbon and then filtering increased the yield in the solution with 0.002 gm. of iron by 32 per cent and in the solution with 0.008 gm. by 12 per cent.

Previously treating the distilled water with carbon increased the growth less in the solution with 0.008 gm. of iron than in the solution with 0.002 gm., possibly because the formation of the larger flocculent precipitate of iron in the former solution acted similarly to carbon in removing injurious substances from the distilled water. Probably the adsorption of the other salts prevented the carbon when added directly to the nutrient

solution from adsorbing to any extent these injurious substances. The slight depression in growth produced by carbon in the solution with 0.002 gm. of iron may well be due to slightly diminishing the amount of soluble iron in the solution by adsorption.

It thus seems possible that the larger flocculent iron precipitate which existed in the solution with 0.008 gm. of iron per liter may have increased growth to some extent by improving the distilled water; but only a small part of the greater growth produced by increasing the iron from 0.002 to 0.008 gm. can be attributed to that cause.

EXPERIMENT 6 (Source of iron in acid, neutral, and alkaline solutions: Ferric chlorid).—The results with ferrous sulphate as the source of iron may well be complicated by the fact that both ferrous and ferric iron doubtless existed in the solution unless the ferrous iron was oxidized by the plants' roots (13). In the very dilute solutions it was, of course, impossible to tell how much of each kind of iron was present. In the following experiment with ferric chlorid as the source of iron naturally ferric iron only was present.

Ferric chlorid was added to the acid, neutral, and alkaline solutions so as to furnish 0.002 and 0.008 gm. of iron per liter. Two seedlings were grown in each flask, six flasks were taken as a unit, and the units triplicated for each treatment. The growth of plants and percentages of iron in the dry substance are given in Table VI.

TABLE VI.—*Comparative weights of rice plants grown in acid, neutral, and alkaline solutions, with ferric chlorid as the source of iron*

Nutrient solution.	Quantity of iron per liter.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—		Iron (Fe_2O_3) in dry stalks and leaves.
						Stalks and leaves.	Whole plant.	
	Gm.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per ct.*
Acid	0.002	1–6	26.50	3.89	0.75	.....	.....	.....
		7–12	32.37	4.59	.87	.....	.....	.....
		13–18	24.96	3.46	.68	3.98	4.75	0.025
Do	.008	19–24	37.67	5.32	.97	.....	.....	.....
		25–30	39.43	5.49	1.10	.....	.....	.....
		31–36	34.62	4.78	.94	5.20	6.20	.026
Neutral	.002	37–42	29.72	4.16	.83	.....	.....	.....
		43–48	28.22	3.81	.80	.....	.....	.....
		49–54	29.56	3.83	.85	3.93	4.76	.022
Do	.008	55–60	38.12	5.17	1.03	.....	.....	.....
		61–66	41.76	5.82	1.21	.....	.....	.....
		67–72	40.70	5.65	1.16	5.55	6.68	.026
Alkaline	.002	73–78	6.96	1.09	.32	.....	.....	.....
		79–84	6.97	1.10	.33	.....	.....	.....
		85–90	6.33	.97	.31	1.05	1.37	.022
Do	.008	91–96	10.03	1.51	.42	.....	.....	.....
		97–102	9.26	1.36	.37	.....	.....	.....
		103–108	7.81	1.22	.33	1.36	1.73	.023

The color of the plants during growth was as follows: At 10 days plants 19 to 36 and 55 to 72 were a good green, 1 to 18 were a lighter green, 37 to 54 were a yellowish green, 73 to 108 had a still poorer color; at 20 days plants 19 to 36 and 55 to 72 were a fair green, 1 to 18 and 37 to 54 were markedly chlorotic, 73 to 108 were more chlorotic; at 30 days plants 19 to 36 and 55 to 72 were of slightly poor color, in 1 to 18 and 37 to 54 color was improved though still chlorotic, 73 to 108 were strongly chlorotic. The color and growth of plants were, on the whole, noticeably inferior to that of plants grown with ferrous sulphate as the source of iron.

The growths made in the acid and neutral solutions were approximately equal and much superior to those in the alkaline solution. Growth was markedly increased in all three solutions by increasing the iron from 0.002 to 0.008 gm. per liter.

The results differ from those of previous experiments with ferrous sulphate because in acid and neutral solutions growth was increased less by increasing the iron from 0.002 to 0.008 gm.; with 0.002 gm. of iron growth was much less in the alkaline solution relative to growth in neutral and acid solutions; increasing the iron from 0.002 to 0.008 gm. in the alkaline solution measurably increased growth instead of markedly depressing it.

The percentages of iron in the dry substance of stalks and leaves varied very little between plants from the different solutions. In each case plants grown in solutions with 0.008 gm. of iron per liter contained very slightly more iron than those grown in similar solutions with 0.002 gm. of iron. It will be noted that there was not such a marked increase in the assimilation of iron from the acid solution as in tests with ferrous sulphate.

EXPERIMENT 7 (Source of iron in acid, neutral, and alkaline solutions: Ferric citrate).—Ferric citrate as a source of iron was compared with ferrous sulphate in experiments 3 and 4, but only 0.002 gm. of iron per liter was used. In this test both 0.002 and 0.008 gm. of iron per liter were compared in the different solutions.

Three seedlings were grown in each flask, but 200 c. c. flasks were used only during the first 15 days, 500 c. c. flasks during the next 15 days, and 1,000 c. c. flasks during the last 10 days of growth. Six flasks were taken as a unit and the units duplicated for each treatment. The growth of plants and percentages of iron in dry stalks and leaves are shown in Table VII.

During the first 30 days all plants were of a good green color, except No. 61 to 72, which were yellowish green. All plants had a good color during the last 10 days.

As in the previous tests with ferric citrate, the growth was equal in acid, neutral, and alkaline solutions with 0.002 gm. of iron per liter. With 0.008 gm. of iron per liter growth was practically equal in the acid and neutral solutions, but much less in the alkaline. Growth was

markedly increased in acid and neutral solutions, but was unaffected in the alkaline solution by increasing the iron from 0.002 to 0.008 gm. per liter.

TABLE VII.—*Comparative weights of rice plants grown in acid, neutral, and alkaline solutions, with ferric citrate as the source of iron*

Nutrient solution.	Quantity of iron per liter.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—		Iron (Fe_2O_3) in dry stalks and leaves.
						Stalks and leaves.	Whole plant.	
	Gm.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per ct.*
Acid	0.002	1–6	87.90	11.02	2.13	.....	.....	.....
		7–12	87.01	10.96	2.15	10.99	13.13	0.019
Do	.008	13–18	124.45	17.42	3.28	.....	.....	.....
		19–24	143.40	18.08	3.65	17.75	21.27	.025
Neutral	.002	25–30	87.31	11.19	2.23	.....	.....	.....
		31–36	84.43	11.08	2.28	11.14	13.40	.016
Do	.008	37–42	130.95	16.35	3.27	.....	.....	.....
		43–48	110.12	13.73	2.60	15.04	17.98	.020
Alkaline	.002	49–54	91.16	11.55	2.34	.....	.....	.....
		55–60	88.65	11.28	2.55	11.42	13.87	.016
Do	.008	61–66	70.30	8.82	2.11	.....	.....	.....
		67–72	91.64	11.75	2.82	10.29	12.76	.020

The percentages of iron in plants grown in acid solutions were slightly greater than in plants grown in neutral and alkaline solutions. The iron contents of plants from all three solutions were slightly increased by increasing the iron from 0.002 to 0.008 gm. per liter.

EXPERIMENT 8 (Source of iron, in acid, neutral, and alkaline solutions: Ferric tartrate).—This experiment was conducted to see whether other organic iron compounds would show the same availability as ferric citrate. The two quantities of iron from ferric tartrate were used in the three nutrient solutions. Two plants were grown in each flask, six flasks were taken as a unit, and the units triplicated for each treatment. The growth of plants and percentages of iron in the stalks and leaves are given in Table VIII.

During the growth the plants were all of good color except No. 37 to 54 and 73 to 90, which were slightly yellowish from the fifteenth to thirtieth day, but of good color the rest of the time.

With 0.002 gm. of iron per liter growth was considerably better in the acid solution than in the neutral or alkaline. With 0.008 gm. of iron per liter growth was practically equal in all three solutions, though possibly there was a slight depression in the neutral solution. Increasing the iron did not increase growth in the acid solution, but did increase it in the neutral and alkaline solutions to a small extent.

The percentages of iron in plants grown in the acid solutions were very slightly greater than in plants grown in the neutral and alkaline solutions.

The iron contents of all plants except those grown in the alkaline solution were increased by increasing the iron from 0.002 to 0.008 gm. of iron per liter.

TABLE VIII.—*Comparative weight of rice plants grown in acid, neutral, and alkaline solutions, with ferric tartrate as the source of iron*

Nutrient solution.	Quantity of iron per liter.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—		(Fe_2O_3) in dry stalks and leaves.
						Stalks and leaves.	Whole plant.	
	Gm.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per ct.*
Acid	0.002	1– 6	67.81	8.97	2.20	.....	.....	.....
		7– 12	56.61	7.92	1.93	.....	.....	.....
		13– 18	62.48	8.65	2.04	8.51	10.57	0.022
Do	.008	19– 24	52.05	6.89	1.90	.....	.....	.....
		25– 30	67.27	8.91	2.19	.....	.....	.....
		31– 36	68.46	9.14	2.26	8.31	10.43	.030
Neutral	.002	37– 42	53.58	7.01	1.52	.....	.....	.....
		43– 48	47.95	6.41	1.47	.....	.....	.....
		49– 54	51.80	6.91	1.58	6.78	8.30	.020
Do	.008	55– 60	57.36	7.58	1.81	.....	.....	.....
		61– 66	56.86	7.72	1.78	.....	.....	.....
		67– 72	63.70	8.60	2.01	7.97	9.84	.025
Alkaline	.002	73– 78	50.22	6.75	1.84	.....	.....	.....
		79– 84	48.17	6.52	1.92	.....	.....	.....
		85– 90	43.01	6.06	1.70	6.44	8.26	.019
Do	.008	91– 96	58.95	8.04	2.40	.....	.....	.....
		97–102	63.13	8.56	2.32	.....	.....	.....
		103–108	62.90	8.46	2.17	8.35	10.65	.022

EXPERIMENT 9 (Source of iron in acid and neutral solutions: Dialyzed iron).—Dialyzed iron was tested as a source of iron in acid and neutral solutions. From the previous experiments it seemed evident that this form of iron would be of very low availability, but as it has been recommended as a source of iron in certain nutrient solutions it was thought advisable to make the test. The comparison between dialyzed iron and ferrous sulphate in the neutral solution was carried out at one time and the comparison between the neutral and acid solutions with dialyzed iron at another time. The two tests are combined in one table for conciseness.

Two plants were grown in each flask, six flasks were taken as a unit, and the unit duplicated for each treatment. Plants in flasks 49 to 84, the comparison between acid and neutral solutions, were grown but 25 days; all others were grown 40 days. The data of growth are given in Table IX.

All plants except No. 25 to 48 were markedly chlorotic at all times.

Dialyzed iron apparently afforded practically no available iron when used in the neutral solution at the rate of either 0.002 or 0.008 gm. of iron per liter. In the acid solution it was slightly more available than in the neutral, although utterly inadequate for the needs of the plant.

TABLE IX.—*Comparative weights of rice plants grown in acid and neutral solutions, with dialyzed iron as the source of iron*

Nutrient solution.	Quantity of iron, per liter.	Source of iron.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—	
							Stalks and leaves.	Whole plant.
				Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Neutral	0.002	Dialyzed iron	1–6	0.67	0.15	0.054	.....	.....
			7–12	.61	.16	.054	0.16	0.21
Do	.008	do	13–18	.63	.17	.054	.....	.....
			19–24	.61	.16	.054	.16	.22
Do	.002	Ferrous sulphate.	25–30	62.19	8.27	1.86	.....	.....
			31–36	65.07	8.48	1.92	8.38	10.27
Do	.008	do	37–42	128.62	16.31	3.73	.....	.....
			43–48	137.50	16.73	4.01	16.52	20.39
Do	.008	Dialyzed iron	49–54	.90	.16	.062	.....	.....
			55–60	.99	.19	.062	.18	.24
Acid	.008	do	73–78	4.38	.67	.182	.....	.....
			79–84	4.26	.64	.176	.66	.84

EXPERIMENT 10 (Effect of applying ferrous sulphate to leaves of plants grown in solutions where iron was markedly unavailable).—In experiment 4 rice failed to grow appreciably in the alkaline solution when 0.008 gm. of iron per liter from ferrous sulphate was used, although it made a fair growth with 0.002 gm. of iron, and in the previous experiment no perceptible growth was made in the neutral solution with dialyzed iron. In order to be certain that the inability of the plants to grow in these solutions was due to a lack of available iron, the following test was made of applying iron to the leaves.

The leaves of plants in these two solutions were brushed with ferrous sulphate, and two control lots were not brushed. The specially treated plants were brushed once with a 0.1 per cent solution of ferrous sulphate, twice with a 0.2 per cent solution, and three times with a 0.4 per cent solution. This was done so that no iron could get from the leaves to the solution. Two plants were grown in each flask, six flasks were taken as a unit, and the units triplicated for each treatment. The plants were grown but 25 days, as results were then decisive, the unbrushed plants having ceased to grow perceptibly some time before. The data on growth are given in Table X.

The leaves brushed with the ferrous-sulphate solution became green in two or three days. New leaves appearing at intervals between the brushings were strongly chlorotic, but became green quickly when treated with the solution. Plants 1 to 12 and 25 to 36, ones that were not brushed at any time, were always strongly chlorotic and made no appreciable growth. The comparative growths of the brushed and unbrushed plants show decisively that the inability of plants to grow in these solutions was due to a lack of available iron.

TABLE X.—*Comparative weights of rice plants grown in two solutions where the iron was unavailable, but with the leaves treated with ferrous sulphate*

Nutrient solution.	Quantity of iron per liter.	Source of iron.	Treatment.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—	
								Stalks and leaves.	Whole plant.
	Gm.				*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Alkaline..	0.008	Ferrous sulphate.	Leaves not brushed..	1-6	2.47	0.39	0.131	....	....
				7-12	2.48	.39	.126	0.39	0.52
Do.....	.008	..do.....	Leaves brushed with ferrous sulphate.	13-18	14.63	2.14	.630	....	....
				19-24	15.95	2.32	.602	2.23	2.85
Neutral ...	.008	Dialyzed iron.	Leaves not brushed..	25-30	.90	.16	.062	....	....
				31-36	.99	.19	.062	.18	.24
Do.....	.008	...do.....	Leaves brushed with ferrous sulphate.	37-42	11.44	1.69	.463	....	....
				43-48	9.40	1.40	.383	1.50	1.92

EXPERIMENT 11 (Effect of increasing the phosphates in the neutral solution).—Since there is always more or less iron in the nutrient solutions precipitated as phosphates, it was important to see whether increasing the phosphates in the solution would affect the growth of plants. In this test the neutral solution used in the preceding experiments was compared with a solution which contained double the quantity of mono- and dibasic potassium phosphate, but which was otherwise similar. Two tests were run at different times, one using ferric chlorid as the source of iron and one using ferric citrate. For the sake of conciseness they are combined in one table.

In the test with ferric chlorid two plants were grown in each flask, 6 flasks were taken as a unit and the units triplicated for each treatment. In the test with ferric citrate one plant was grown in each flask, 12 flasks were taken as a unit, and the units duplicated for each treatment. The data on the growth of these plants are given in Table XI.

The ferric-chlorid plants were all of rather poor color, those with 0.002 gm. of iron per liter being poorer than those with 0.008 gm., but no differences were apparent between plants in the ordinary and double-phosphate solutions. The ferric-citrate plants in both ordinary and double-phosphate solutions were of good color at all times.

In regard to growth and iron content, there were no appreciable differences between plants in the ordinary and double-phosphate solutions, although the very slight differences in growth were always in favor of the ordinary solution.

TABLE XI.—*Comparative weights of rice plants grown in neutral solution with phosphates doubled, with ferric chlorid or citrate source of iron*

Nutrient solution.	Quantity of iron per liter.	Source of iron.	Flasks no.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—		Iron (Fe_2O_3) in dry stalks and leaves.
							Stalks and leaves.	Whole plant.	
				Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per ct.*
Neutral	0.002	Ferric chlorid	1–6	28.22	3.54	0.68			
			7–12	34.30	4.24	.84			
			13–18	28.76	3.63	.69	3.80	4.54	0.020
Neutral, with double phosphates.	.002	do	19–24	29.84	3.78	.74			
			25–30	27.43	3.49	.68			
			31–36	29.42	3.89	.78	3.72	4.45	.021
Neutral	.008	do	37–42	42.12	5.21	1.20			
			43–48	37.24	4.56	1.03			
			49–54	38.16	4.76	1.10	4.84	5.95	.023
Neutral, with double phosphates.	.008	do	55–60	39.23	4.88	.99			
			61–66	37.30	4.72	.93			
			67–72	34.51	4.39	.90	4.66	5.60	.022
Neutral	.008	Ferric citrate	1–12	202.36	29.95	7.41			
			13–24	192.20	28.72	7.11	29.34	36.65	.019
Neutral, with double phosphates.	.008	do	25–36	181.78	27.33	6.74			
			37–48	189.34	28.85	7.21	28.09	35.07	.021

SOLUBILITY OF IRON IN THE NUTRIENT SOLUTIONS

An attempt was made to estimate the soluble iron in some of the nutrient solutions by filtering the solutions to remove precipitated iron and determining iron in the filtrate. Solutions 1 to 9 (used in experiment 4) were analyzed after they had stood various lengths of time both with and without plants growing in them. Iron in all the solutions was determined by the colorimetric method with potassium sulphocyanate, but solutions 1, 2, 4, 5, 7, and 8 without the growth of plants were also analyzed by the method of Stokes and Cain. Practically the same results were obtained by the two methods. The results are shown in Table XII. Figures for iron in the solutions without plants are the averages of two or more determinations, while the figures for iron in solutions in which plants had grown represent single determinations. The results for solution 5 without plants are not trustworthy, as this solution could not be filtered absolutely clear by any device the writers considered permissible to employ.

In a comparison of the growth of plants in the different solutions with the amount of iron in the filtered solutions, almost as many discrepancies as agreements are apparent. One striking discrepancy is the small amount of iron in the filtrate of No. 3 and the good growth of plants in this solution. Repeated analyses of the precipitate left on the filter

showed that practically all the iron added to this solution was precipitated. As this solution evidently had less precipitate than some others, the character of the precipitate probably varied somewhat in the different solutions. The amount of iron present in the filtered solutions in which plants had grown also failed to correspond to the growth of plants.

TABLE XII.—*Quantity of iron (in grams) in filtered solutions at different times after addition of iron to solution, with and without growth of plants in the solution*

Solution No.	Nutrient solution.	Source of iron.	Iron per 1,000 liters.	Quantity of iron in 1,000 liters of filtered solution.							
				No plants in solutions.			Plants grown in solutions.				
				24 hours after iron had been added.	72 hours after iron had been added.	120 hours after iron had been added.	After 10-day-old plants had been grown in solution for 96 hours.	After 14-day-old plants had been grown in solution for 96 hours.	After 30-day-old plants had been grown in solution for 48 hours.	After 35-day-old plants had been grown in solution for 48 hours.	After 37-day-old plants had been grown in solution for 96 hours.
1	Acid	Ferrous sulphate	2.0	1.35	1.30	0.90	0.12	0.07	0.05	0.05	0.03
2	do	do	8.0	5.00	4.20	3.70	.30	.30	.43	.23	.07
3	do	Ferric citrate	2.0	< .40	.0	.0	.12	< .03	.07	.05	.03
4	Neutral	Ferrous sulphate	2.0	1.35	1.30	1.00	.67	.49	1.27	1.13	.80
5	do	do	8.0	1.00	.70	.50	.07	.03	.17	.10	.07
6	do	Ferric citrate	2.0	1.65	1.20	1.00	.80	.87	1.63	1.33	.80
7	Alkaline	Ferrous sulphate	2.0	1.15	.80	.40	.37	.40	1.33	.86	.60
8	do	do	8.0	.....	.....	.03	< .07	.05	.18	.....	.....
9	do	Ferric citrate	2.0	1.40	.90	.40	.57	.57	1.00	.93	.53

The growth of plants did not agree with the quantity of iron found in the filtered solutions because, without doubt, colloidal iron, as well as truly soluble iron, was present. Previous work having shown that colloidal iron was not available for rice (6), the determinations in Table XII did not represent the available iron. At this great dilution it was, of course, impossible to distinguish analytically between colloidal and soluble iron or between ferrous and ferric iron. While the existence of colloidal iron could not be definitely demonstrated in these solutions, it is well known that most ferric salts in dilute solutions are more or less completely hydrolytically dissociated into colloidal ferric hydroxid and the acid. Moreover, a test showed that dialyzed iron could not be distinguished from distilled water with regard to color or filtration when used in somewhat greater concentration than iron was present in the above filtrates.

In solutions where ferric iron was used iron was probably present in the following forms: (1) As precipitated ferric phosphate and hydroxid, (2)

as colloidal ferric hydroxid, (3) as soluble undissociated iron compounds, and (4) as ionized iron. There was probably a balance between these forms of iron, as after filtering the nutrient solutions more precipitate formed on standing a short time.[1] From determinations of iron in the filtered nutrient solutions it is evident that more or less half the iron was precipitated as phosphate and hydroxid. The amount and composition of this precipitate probably varied somewhat in the different solutions (9). The greater part of the remaining iron was probably present as colloidal ferric hydroxid. The available iron, which included the soluble undissociated and ionized iron, was undoubtedly extremely small and was governed chiefly by the completeness of the hydrolysis of the dissolved iron.

The amount of iron hydrolized would depend on the reaction of the solution, being less in acid solutions, and would also depend on the form in which iron was added, being less with the less ionized organic salts. The effect of the form of iron and the reaction of the solution on the assimilation of iron by rice is thus easily comprehensible.

While the amount of soluble or available iron could not be determined analytically, some idea of the very small amount present could be obtained by comparing the amount of iron absorbed by the plants with the total amount of iron added in the volume of solution available during growth. This calculation showed that the plants absorbed only one-fifteenth to one two-hundredth part of the iron supplied, even in solutions where growth was obviously inhibited by lack of iron. If it is assumed that one fifteenth to one two-hundredth part of the iron added to the solutions was in a soluble condition, the concentration of the soluble iron in some cases would have been from 0.13 to 0.01 part per million. A more probable assumption is that the concentration of soluble iron present at any one time was even lower and that as iron was removed by the plant more went into solution. It thus appears that in some cases the amount of iron in true solution must have been too small for slight differences to be accurately determined. It is evident that in certain nutrient solutions rice can assimilate sufficient iron when the concentration of soluble iron is probably less than 1 part in 10,000,000.

Comparative analyses in Table XII of solutions in which plants had and had not grown show that at least the amount of colloidal iron was notably diminished by the growth of plants in the solution. The colloidal iron was evidently precipitated.

SUMMARY OF EXPERIMENTAL RESULTS

The results of the previous culture experiments showed plainly that rice in nutrient solutions was not particularly sensitive to an acid or alkaline reaction *per se*. Apparently the reaction of the nutrient solution affected the growth of rice only through influencing the availability of the

[1] The precipitates in these dilute nutrient solutions were iron compounds, as before the addition of iron salts the solutions were perfectly clear.

iron, since the relative growths made in acid, neutral, and alkaline solutions depended on the kind and quantity of iron supplied. This is well shown by Table XIII, which summarizes the relative growths of stalks and leaves made in the three solutions with different kinds and quantities of iron. The growth of stalks and leaves [1] made in the acid solution is always taken as 100; growths in the neutral and alkaline solutions are expressed relative to 100.

TABLE XIII.—*Relative growths of rice plants in acid, neutral, and alkaline solutions with different sources and amounts of iron*

Source of iron in nutrient solutions.	Iron per liter added to nutrient solutions.	Relative growths in—			Table from which data were calculated.
		Acid solution.	Neutral solution.	Alkaline solution.	
	Gm.				
Ferrous sulphate	0.002	100	88	.....	II
Do	.....	100	74	51	III
Do	.....	100	95	95	IV
Do	.008	100	105	.....	I
Do	.004				
Do	.002	100	107	.....	II
Do	.008	100	132	.....	II
Do	.....	100	111	2	IV
Ferric chlorid	.002	100	99	26	VI
Do	.008	100	107	26	VI
Ferric citrate	.002	100	85	86	III
Do	.....	100	94	97	IV
Do	.....	100	101	104	VII
Do	.008	100	85	58	VII
Ferric tartrate	.002	100	80	76	VIII
Do	.008	100	96	100	VIII
Dialyzed iron	.008	100	27	.....	IX

It is evident that with 0.002 gm. of iron per liter, growth was more or less best in the acid solution, while with 0.008 gm. of iron per liter growth was best in the neutral solution with some forms of iron. Growth with most, but not all, forms and quantities of iron was strikingly inferior in the alkaline solution.

The effect on growth of increasing the quantity of iron in the different solutions is significant in throwing light on the availability of the iron in these solutions. In Table XIV are shown the extents to which growth was increased or decreased by increasing the iron in the three solutions from 0.002 to 0.008 gm. per liter. The growth made in each solution (acid, neutral, or alkaline) with 0.002 gm. of iron per liter is taken as 100, and the growth made with 0.008 gm. of iron per liter in these solutions is expressed relative to 100.

[1] In this and previous comparisons more significance was attached to the weight of stalks and leaves than to the weight of the whole plant. The weights given for the roots must have been very slightly in excess of the true values, as the roots were always more or less contaminated with a precipitate that could not be removed by washing. Also, the root growth relative to top growth is markedly influenced in some cases by an insufficiency of a mineral nutrient.

TABLE XIV.—*Relative growths of rice plants with 0.002 and 0.008 gm. of iron per liter in the different solutions*

Source of iron in nutrient solution.	Growth [a] with 0.008 gm. of iron per liter in—			Table from which data were calculated.
	Acid solution.	Neutral solution.	Alkaline solution.	
Ferrous sulphate	141	212	.....	II
Do	179	209	4	IV
Do	.....	197	.....	V
Ferric chlorid	131	141	140	VI
Do	.....	127	.....	XI
Dialyzed iron	.....	100	.....	IX
Ferric citrate	162	135	90	VII
Ferric tartrate	98	118	130	VIII

[a] Growth with 0.002 gm. of iron per liter=100.

In most cases an increase in the amount of iron added to the solution produced a marked increase in growth. With ferric tartrate in the acid solution an increase in iron produced no increase in growth, as apparently the smaller quantity of iron was adequate. In the alkaline solution with ferric citrate and with ferrous sulphate there were, respectively, no increase and a striking depression in growth, following an increase in the amount of iron added to the solution. Increasing the ferric citrate in this solution apparently did not increase the soluble iron and increasing the ferrous sulphate must have decreased it, as the smaller amounts of iron were also inadequate in these solutions.

From the growth of the plants it appears that ferrous sulphate, ferric citrate, or ferric tartrate used in proper quantities afforded sufficient iron in acid or neutral solutions. With the two quantities of iron used ferric chlorid was inferior as a source of iron, and dialyzed iron was utterly inadequate. Ferric tartrate was the only form of iron tried which appeared to afford sufficient iron in the solution alkaline with calcium carbonate.

The addition of carbon black to the neutral nutrient solution with 0.002 gm. of iron per liter very slightly depressed growth, but carbon black in the solution with 0.008 gm. of iron did not affect the yield. Treating the distilled water with carbon and filtering previous to its use in the nutrient solution increased growth considerably over that in the ordinary nutrient solution.

Doubling the phosphates in the neutral nutrient solution did not measurably affect growth when either ferric chlorid or citrate was used as the source of iron.

In all the cultural tests, plants grown in acid solutions contained more iron than those grown in corresponding neutral or alkaline solutions. Plants grown in neutral solutions contained more iron than those grown

in corresponding alkaline solutions when ferrous sulphate or ferric chlorid was the source of iron, but about equal percentages when ferric citrate or tartrate was the source of iron. With ferric chlorid, citrate, or tartrate an increase in the quantity of iron from 0.002 to 0.008 gm. of iron per liter in acid, neutral, or alkaline solution raised the percentage of iron in the plant. Increasing the ferrous sulphate in the acid solution did not increase the percentage of iron in the plants, while in the neutral solution it did have this effect. The percentages of nitrogen, phosphoric acid, lime, magnesia, and carbon-free ash in plants grown in six different solutions did not vary appreciably. The relative percentages of iron in the plants thus agreed with relative growths in showing that the amount of available iron in most solutions was the main factor controlling growth.

In regard to the percentages of iron in the plants two anomalous features are apparent in the preceding tables: (1) The percentages of iron in plants supplied with ferrous sulphate were higher than in plants supplied with ferric citrate, and (2) in two cases plants which made no growth because of a lack of iron contained as high a percentage of iron as plants of good growth.

Experiments 3 and 4 each afford a comparison of ferric citrate and ferrous sulphate in the three different nutrient solutions. In all cases 0.002 gm. of iron per liter from ferric citrate produced a better growth of plants with a lower percentage of iron than did the same quantity of iron from ferrous sulphate. As tests 2, 3, 4, and 7 show, neither of these forms of iron, supplied at this rate, furnished sufficient iron for the maximum needs of the plant. Therefore the higher percentages of iron in the ferrous-sulphate plants could not have been due to excessive consumption. About the only explanation of the anomaly that occurs to the writers is that the ferrous-sulphate plants contained a certain amount of iron which was ineffective in the metabolism of the plant. In the solutions to which ferrous sulphate was added undoubtedly both ferrous and ferric iron existed in solution, while in the solutions with ferric citrate there was only ferric iron. It is possible that in the solutions with ferrous sulphate the plants absorbed both ferrous and ferric iron and that the ferrous iron was not so effective in the plant as the ferric iron. The ferrous-sulphate plants might therefore contain a certain amount of effective and non-effective iron. This explanation is somewhat doubtful, as it is not, to the knowledge of the writers, supported by similar well-established facts.

The second irregularity is the high percentage of iron in plants 1 to 12, experiment 1, and plants 85 to 96, experiment 4, the former being grown without the addition of any iron to the solution, and the latter obtaining practically no iron from the alkaline solution, as proved by experiment 10. It is, of course, possible that the high percentages of iron were due to contamination, but this is thought not to be the cause. The plants made practically no growth and were so strongly chlorotic that they

could have elaborated scarcely any organic matter; the leaves were especially thin and often withered as soon as formed. It is possible that the high percentage of iron in the dry substance might have been due to the fact that enough iron was not present in the plant to start the production of carbohydrates, which would have lowered the percentage. While this explanation opens up several points not covered by the present investigation, it is supported somewhat by the following test (Table XV, first test), where plants grown for 13 days with iron and then for 13 days without iron contained a lower percentage of iron than plants grown the full 26 days without iron.

TABLE XV.—*Percentages of iron in rice plants grown with and without iron*

Test and treatment of plants.	Average dry weight of stalks and leaves per plant.	Percentage of iron (Fe_2O_3), in dry stalks and leaves.
First test:		
Grown for 13 days, without the addition of iron to the solution	*Gm.* 0.017	0.020
Grown for 26 days, without the addition of iron to the solution	.018	.026
Grown for 13 days, without the addition of iron to the solution and then for 13 days with iron	.097	.020
Grown for 26 days, with the addition of iron to the solution	.311	.027
Second test:		
Grown 13 days, without the addition of iron to the solution	.016	.015
Grown 26 days, without the addition of iron to the solution	.019	.024
Grown 40 days, without the addition of iron to the solution	.026	.049
Grown 40 days, with the addition of iron to the solution	1.547	.030

In the first test plants grown 26 days without iron contained a higher percentage of iron than those grown 13 days, although there was practically no increase in growth. This peculiarity was repeated in a second test (Table XV).

From the regularity of these results it appears they were due to peculiarities in the metabolism of plants grown without iron. That plants grown for 40 days in the iron-free solution made only slightly more growth than those grown for 13 days, although they contained much more iron, is probably due in part at least to the immobility of iron in the plant (7). Considering the extremely low concentration from which plants absorb their iron in solutions supplied with iron, it is probable that the plants obtained traces of iron even from the "iron-free" solutions, as it would be very difficult to exclude one part of iron in several hundred million of solution.[1]

The attempt to determine the soluble iron in the various solutions was unsuccessful because of the impossibility of distinguishing between col-

[1] If the difference between the iron present in plants grown 13 days and 40 days (Table XV) is due to iron absorbed from the solution, 0.0000055 gm. of ferric oxid (Fe_2O_3) was absorbed between the thirteenth and fortieth day by the three plants grown in each flask. As 1,600 c. c. of solution were supplied per flask during this interval, the concentration of soluble iron, expressed as Fe_2O_3, would have been less than 3½ parts in 1,000,000,000.

loidal and soluble iron at relatively great dilutions. From the amount of iron supplied and the amount absorbed it appeared that there could have been not more than approximately 1 part in 10,000,000 in solution at one time and very probably much less. The much larger quantities of iron found in the filtrate of the nutrient solutions were attributed to colloidal iron, which a previous test showed to be unavailable to the plant.

DISCUSSION OF RESULTS

The reactions of the solutions used in this work could not be exactly measured by titration because of the presence of interfering ions. It is evident, however, that the acid solution was relatively quite strongly acid as it contained only monopotassium phosphate besides a trace of sulphuric acid, the neutral solution was nearly neutral as it contained a mixture of mono and di-potassium phosphates, and the alkaline solution was slightly alkaline from the presence of calcium carbonate and its reaction with the phosphates.

The preceding tests demonstrated that the growth of rice was markedly dependent on the quantity and form of iron added to these nutrient solutions and apparently dependent on the reaction of the solution only so far as it affected the availability of the iron. These facts are important in bearing on the nature and cause of lime-induced chlorosis, rice being markedly affected with this nutritional disturbance. Previous work in soil cultures showed pretty decisively that lime-induced chlorosis was not caused by lack of any mineral nutrient except possibly iron, but did not show whether the reaction of the soil in itself affected the plants. The present study seems to substantiate the previous work and show, moreover, that rice is not particularly sensitive to the reaction *per se*, provided the iron supply is maintained. While in soils there are many more factors affecting the availability of iron than in nutrient solutions, the preceding results point strongly to calcium carbonate diminishing the quantity of available iron in a soil through affecting the reaction. Direct evidence on this latter point is afforded by the results of Morse and Curry (11) and Ruprecht and Morse (12). The results also point to different iron compounds, particularly the organic and inorganic compounds, varying greatly in their availability in calcareous soils. The existence of organic iron compounds in the soil has been pointed out by Hartwell and Kellogg (8).

While all the tests reported here were carried out with rice, certain results are of general interest in relation to the proper composition of plant-nutrient solutions. The extent to which growth was dependent on the iron supply in the previous tests shows how important it may be to consider the form and quantity of iron used in the nutrient solution. Evidently the addition of a few drops of a dilute iron solution, as recommended in most plant physiologies, may not insure an adequate supply of iron. While the color of the leaves will indicate a marked deficiency of iron, a slight deficiency may materially diminish the yield without mate-

rially affecting the appearance of the plants. It is, therefore, not sufficient to judge the adequacy of the iron supply by the mere color of the leaves. Also a sufficiency of iron can not be insured by simply increasing the quantity of iron added to the solution as this may even diminish the amount of available iron.

Although this work demonstrated chiefly the reaction of the solution and the source of iron as factors influencing the availability of iron, the importance of other conditions was indicated. Undoubtedly the frequency with which the nutrient solution is changed influences appreciably the availability of the iron. The results in Table XII showed that the precipitated iron increased with the age of the solution and also with the growth of plants in the solution. This increase in precipitated iron probably accompanied a certain decrease in soluble iron, as there was doubtless some balance between the precipitated, colloidal, and soluble iron.

Doubtless absorption also affected the quantity of soluble iron in certain cases. The diminution in growth following the addition of carbon black to the solution with 0.002 gm. of iron per liter in experiment 10 was probably caused by a decrease in soluble iron through simple adsorption. In experiment 4 the decrease in available iron following an increase in the quantity of iron added to the alkaline solution may have been partially due to the adsorption of soluble iron by the larger precipitate of iron.

On the basis that hydrolysis was the chief factor determining the amount of soluble iron in the solutions, one would not expect that increasing the phosphates in the neutral solution would appreciably affect the iron assimilated by the plants. Experiment 11 confirmed this.[1]

This work furnished no evidence of rice being able to assimilate other than soluble iron, but tended to confirm previous work showing that even colloidal iron is unavailable (6). It did show, however, that rice used iron, which must have been present in exceedingly low concentrations.

The facts established concerning the availability of iron in these nutrient solutions help explain results obtained by certain investigators with other nutrient solutions. It is realized, however, that the availability of iron in each nutrient solution probably varies according to the concentration and composition of the solution as well as according to the method of conducting the cultural test.

The chlorosis of peas in certain nutrient solutions observed by Mazé et al. (10) was evidently not due to the excretion of calcium carbonate by the roots, but to salts in the solution depressing the availability of the iron. Probably the potassium silicate, which is strongly hydrolyzed into potassium hydroxid, was chiefly responsible for the nonavailability of the iron. The chlorosis observed by Von Crone (3) with plants in certain nutrient solutions was not due to the soluble phosphates, but probably to a deficiency of iron. In certain solutions the lack of iron was

[1] If the concentration of phosphate ions had been very low, such as would be afforded by tricalcium or ferric phosphate, doubtless the addition of soluble phosphates would have depressed the available iron.

doubtless due to the reaction of the solution, calcium carbonate being present. In other solutions where ferric phosphate was the source of iron, addition of soluble phosphates produced chlorosis. The soluble phosphates here evidently precipitated the very small amount of iron that went into solution from the decomposition of ferric phosphate. Von Crone made the mistake of assuming that because ferric or ferrous phosphate furnished sufficient iron in some solutions that it did in all solutions. Doubtless the reason that ferrous phosphate furnished sufficient iron in Von Crone's solution was due to the fact that the concentration of phosphate ions was also particularly low.[1]

The results of this work, as well as that of Takeuchi (15) and Benecke (1), showed that soluble phosphates do not in themselves produce chlorosis. Benecke in his criticism of Von Crone's solution failed to take into account that part of the iron he determined in his tests of the solubility of iron phosphates was colloidal iron.

SUMMARY

Rice was grown in acid, neutral, and alkaline solutions with different forms and quantities of iron to determine whether rice is particularly sensitive to the reaction of the solution and whether the reaction of the solution influences the assimilation of iron.

In nearly all cases growth was much better in the nutrient solutions employed with 0.008 gm. of iron per liter than with 0.002 gm. When judged by the growth of plants ferrous sulphate, ferric citrate, and ferric tartrate afforded sufficient iron when used in proper quantities in the acid and neutral solutions. Ferric chlorid was an inferior source of iron, and dialyzed iron utterly inadequate. Only ferric tartrate furnished sufficient iron in the alkaline solution.

Plants grown in the acid solutions contained the highest percentages of iron. Plants grown in the neutral solutions contained higher percentages of iron than those grown in the alkaline solutions when some forms of iron were used, but equal percentages when other forms of iron were used. The percentages of nitrogen, phosphoric acid, lime, magnesia, and carbon-free ash in plants grown in six different solutions did not vary appreciably when compared with the iron content.

It was evident that rice was not particularly sensitive to the reaction of the solution, except as the reaction influenced the availability of the iron. This substantiates previous work in showing that lime-induced chlorosis is caused by a lack of iron and indicates strongly that the only action of carbonate of lime in inducing chlorosis lies in diminishing the availability of the iron.

The amount of available iron in the different solutions could not be determined analytically, because of the impossibility of distinguishing

[1] Ferrous and ferric phosphate evidently afford iron and phosphoric acid not through the dissolving action of plant roots, as Crone believed, but through the hydrolytic decomposition of these compounds. As a result of this decomposition, colloidal iron hydroxid is formed, as well as phosphate ions and a very small amount of soluble iron (9, 2).

between colloidal and soluble iron. Calculations showed, however, that the concentration of available iron in many cases must have been less than one part in 10,000,000 of solution

Reference was made to the bearing of these results on the proper composition of plant nutrient solutions.

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INFLUENCE OF BORDEAUX MIXTURE ON THE RATES OF TRANSPIRATION FROM ABSCISED LEAVES AND FROM POTTED PLANTS

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INTRODUCTION

Since the introduction of Bordeaux mixture much work has been done on the effects which this fungicide has on the growth of normal plants. For more than 20 years this work has occupied the attention of pathologists. During this time progress has been made, but there still remains much to be done. Many observations have been made as to the effect which an application of this spray has upon transpiration rates, and a review of the literature brings out the fact that the conclusions drawn from these observations are conflicting.

Rumm (15)[2] found that when abscised leaves were placed in water the unsprayed ones wilted first; from this he concluded that there is a decrease in the rate of transpiration following an application of the spray. Clinton (4) expressed the view that the water pores and stomata of potato leaves are clogged by the spray and as a result transpiration is decreased. Schander (16), Bayer (2), and Müller-Thurgau (14) each expressed the opinion that decreased transpiration rates follow spraying with Bordeaux mixture.

As a result of extensive investigations, Frank and Kruger (8, 9) conclude that the water loss from sprayed plants is greater than from plants not so treated. Bain (1) likewise found that an increased rate of transpiration occurs in peach seedlings as a result of spraying. He arrived at this conclusion from the fact that he found it necessary to supply water more frequently to the roots of seedlings that had been sprayed. More recently, however, Duggar and Cooley (6, 7) have furnished direct evidence bearing upon this question. In a series of very carefully performed experiments they have demonstrated that not only does a film of Bordeaux mixture on the leaves of castor beans, tomatoes, and potatoes increase their rates of transpiration but that other surface films have a similar effect. They have brought out the fact that certain specific qualities of the films applied are definitely related to the phenomenon of increased transpiration. They further state that the color of the film applied is also a factor to be considered in this connection.

[1] Thanks are due to Dr. M. T. Cook for suggesting the problem and to Dr. J. W. Shive for valuable suggestions during the progress of the work and for aid and criticism in the preparation of the manuscript.

[2] Reference is made by number to "Literature cited," p. 547–548.

While the last-named authors have definitely established the fact that surface films with certain specific characteristics have an accelerating influence on rates of transpiration when applied to the leaves of castor bean, tomato, and potato plants, the problem here involved seemed to be of sufficient importance to warrant further investigation in a different locality and under different conditions.

It was the purpose of the experiments here reported to determine the influence of Bordeaux mixture on the rates of transpiration of abscised leaves of several species, as well as to determine the effect of this spray material upon the rates of water loss from a variety of potted plants. The experiments were carried out in the greenhouse of the Department of Plant Pathology of the New Jersey Agricultural Experiment Station.

EXPERIMENTS WITH ABSCISED LEAVES

EXPERIMENTAL METHODS.—Abscised leaves of radish (*Raphanus sativus* L.), bean (*Phaseolus vulgaris* L.), Swiss chard (*Beta cycla* L.), *Hibiscus cardinalis*, *Clerodendrum balfouri*, *Caladium* sp., *Datura meteloides*, and castor bean (*Ricinus communis* L.) were used. The leaves, together with portions of the stems, were severed from the plants and the cut ends immediately placed in water. They were then taken to the greenhouse room, where the experiments were carried out. The leaves were now cut off under water and the petioles were inserted into Erlenmeyer flasks having a capacity of 180 c. c.; the flasks were nearly filled with water. A layer of cotton was then placed tightly around the leaf petiole just at the surface of the water; this served to hold the leaf in place. The flasks were sealed by pouring melted wax over the cotton around the leaf petioles. This wax was prepared according to the formula of Briggs and Shantz (3) and consisted of a mixture of about 80 per cent of paraffin and 20 per cent of petrolatum. The mixture had a melting point of about 45° C. By pouring a layer of this wax about 1 cm. thick on the layer of cotton around the leaf petioles the leaves were held firmly in place. To permit the entrance of air into the flasks as water was removed by transpiration from the leaf a small hole was made in the wax with a pin.

In testing the effect of Bordeaux mixture on the rates of transpiration of abscised leaves, six leaves of each species were employed. These were chosen from larger groups of leaves which had been mounted as above described and allowed to stand for some time in order to become adjusted to the new conditions. Leaves of the different species used were chosen with special reference to equality of surface exposed and also with reference to similarity of general appearance. The six leaves chosen were divided into two groups of three each. For facility in comparison and for ease of reference in discussion, one of these groups will be designated "series A" for periods before treatment and "series A'" for periods after treatment; the other group, remaining untreated throughout the experiment, will be designated "series B."

It is, of course, not possible in experiments of this nature to subject each leaf to precisely the same changes in aerial conditions. Special precautions were taken, however, to arrange the leaves involved in a single experiment in such a manner with reference to each other and to their surroundings that each might experience, as nearly as possible, the same changes in environmental conditions. This was accomplished by placing the flasks in rows 2 feet apart on a table centrally located in the greenhouse, where air currents from ventilators or open doors would affect the plants similarly. The arrangement decided upon for each experiment was maintained until the experiment was terminated. The leaves were removed from their positions only for the purpose of weighing, and each leaf was returned to the position previously occupied as soon as the weighing was completed.

Each flask with its leaf (in series A and series B) was weighed to 0.01 gm., allowed to stand for a definite time period, and again weighed. The difference between the two readings, of course, gave the absolute transpiration for the time period. The first period of exposure, before spraying the leaves of series A with Bordeaux mixture, may be regarded as the standardization period, and the leaves of the control series B may be regarded as the standard leaves for comparison. At the close of the standardization period the leaves of series A, which then became series A′, were sprayed with Bordeaux mixture. This spray was prepared in the usual way and contained 12 gm. of copper sulphate and 12 gm. of lime in 1 liter of the mixture, approximating the 5–5–50 formula of agricultural practice. Both upper and lower surfaces of the leaves were sprayed, the spray being applied with an atomizer. This method yielded a very uniform film of spray over the leaf surfaces.

A comparison of the ratios between the transpiration quantities of series A and those of series B for the time period before spraying with similar ratios for the time period after spraying will determine whether the transpiration rates of the leaves for the periods after spraying have changed. For the sake of convenience in the treatment of ratios, transpiration quantities for series A will be termed "A" for periods before treatment and "A′" for periods after treatment. The corresponding transpiration quantities for series B will be designated "B" throughout, since the leaves of series B were not treated with Bordeaux mixture. If then the ratio of A to B is greater than the ratio of A′ to B, it follows that the rates of transpiration of the sprayed leaves have suffered a decrease relative to the rates of the unsprayed leaves. If, however, the ratio of A to B is less than the ratio of A′ to B, then the rates of transpiration of the sprayed leaves have increased, relative to the rates of the unsprayed leaves. In making such comparisons it must be assumed, of course, that the ratio of A to B for successive time periods would remain unchanged if the leaves of series A were not treated. It could scarcely be expected that the ratio of A to B would remain constant for successive intervals, since it is not possible to subject all the leaves to precisely the same changes in

aerial conditions. Furthermore, internal changes continually taking place in the leaves would tend to cause some variations in the ratio of A to B, since the degree of these changes would certainly vary from leaf to leaf, even supposing the nature of these changes to be the same in all the leaves. It may reasonably be supposed, however, that variations in the ratio of A to B for successive intervals, due to internal changes and to differences in the changes of environmental conditions experienced by the different leaves of a series, are comparatively small. This was, indeed, found to be the case in a series of preliminary experiments. The time period during which an experiment may be conducted with relatively very small variations in the ratio of A to B, owing to conditions other than the treatment with spray, varies, of course, with the different species.

EXPERIMENTAL RESULTS.—The experimental data showing the effect of Bordeaux mixture on the transpiration of abscised leaves are presented in Table I. The first column of this table gives the names of the various species dealt with and the time at the beginning and end of each experimental period. This is followed, under "Periods before treatment," by three columns giving transpiration quantities, in grams, of the three leaves of series A; then are given three columns presenting transpiration data for series B for the same time periods. The last column of this section gives ratios obtained by summing the values of the transpiration quantities of the three leaves (on the same horizontal line) of series A and dividing this summed value by the summed value of the corresponding transpiration quantities for series B. The second section, under "Periods after treatment," presents in the same way as the first the data comparing series A′ with series B. The table is further divided into a number of horizontal sections, each section presenting all of the data for a single species.

The data included in the first horizontal section of Table I represent an experiment extending over a total time period of more than five days. The transpiration data for datura leaves presented in the second horizontal section of the table represent an experiment extending over a total time period of more than four days. During these two experiments weighings were made each day, as indicated. The data presented in the remaining sections of the table represent experiments conducted mainly to determine whether the spray becomes effective in its influence on transpiration, immediately after drying on the leaves, or whether modified rates of water loss begin at some later period. All of these experiments were conducted during the same day, extending over a total time period of a little more than 10 hours. Water loss from each leaf was determined at intervals of two hours. The containers used in these experiments were small, so that weighings could be made to 0.01 gm. At the end of the two standardization periods the leaves of the A series were sprayed on the upper and lower foliar surfaces, and weighings were made soon after the spray had completely dried on the leaves.

TABLE I.—*Effect of Bordeaux mixture on the rates of transpiration of abscised leaves for periods before and after treatment*

PERIODS BEFORE TREATMENT

Plant and period.	Transpiration quantities.						Ratio A : B.
	Series A.			Series B (control).			
	Leaf 1.	Leaf 2.	Leaf 3.	Leaf 1.	Leaf 2.	Leaf 3.	
Ricinus communis:	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
Feb. 1, 8.20 a. m.	6.40	3.60	2.60	1.30	4.50	6.20	1.05
1, 3.30 p. m.							
2, 10.00 a. m.	1.40	2.40	4.00	2.70	2.70	4.60	.78
2, 6.00 p. m.							
3, 8.30 a. m.	1.70	4.20	3.50	2.80	4.80	5.90	.70
3, 4.00 p. m.							
Total	9.50	10.20	10.10	6.80	12.00	16.70	.84
Datura metaloides:							
Mar. 16, 4.00 p. m.	10.90	8.20	1.50	9.50	5.80	7.90	.89
17, 1.25 p. m.							
17, 1.25 p. m.	4.70	4.20	2.20	4.80	4.10	4.60	.85
18, 11.30 a. m.							
Total	15.60	12.40	3.70	14.30	9.90	12.50	.86
Phaseolus vulgaris:							
Mar. 22, 8.50 a. m.	.08	.14	.18	.08	.43	.21	.56
22, 10.40 a. m.							
22, 10.40 a. m.	.07	.14	.21	.13	.18	.20	.82
22, 12.40 p. m.							
Total	.15	.28	.39	.21	.61	.41	.67
Beta cycla:							
Mar. 22, 9.45 a. m.	.17	.27	.23	.23	.45	.25	.72
22, 10.35 a. m.							
22, 10.35 a. m.	.07	.20	.21	.19	.31	.27	.62
22, 1.35 p. m.							
Total	.24	.47	.44	.42	.76	.52	.68
Raphanus sativus:							
Mar. 22, 9.10 a. m.	.17	.25	.....	.10	.30	.17	.72
22, 11.10 a. m.							
22, 11.10 a. m.	.16	.33	.....	.21	.27	.20	.72
22, 12.10 p. m.							
Total	.33	.58	.....	.31	.57	.37	.72
Caladium sp. (small variety):							
Mar. 22, 9.05 a. m.	.30	.28	.30	.33	.29	.21	1.06
22, 10.55 a. m.							
22, 10.55 a. m.	.25	.20	.25	.20	.20	.14	1.29
22, 12.55 p. m.							
Total	.55	.48	.55	.53	.49	.35	1.15
Hibiscus cardinalis:							
Mar. 22, 9.30 a. m.	.04	.07	.08	.12	.10	.05	.73
22, 11.20 a. m.							
22, 11.20 a. m.	.09	.08	.05	.05	.18	.10	.67
22, 1.25 p. m.							
Total	.13	.15	.13	.17	.28	.15	.68
Clerodendron balfouri:							
Mar. 22, 8.35 a. m.	.09	.13	.10	.13	.10	.07	1.06
22, 10.30 a. m.							
22, 10.30 a. m.	.11	.15	.13	.15	.10	.14	1.00
22, 12.30 p. m.							
Total	.20	.28	.23	.28	.20	.21	1.02
Datura metaloides:							
Mar. 22, 8.15 a. m.	.66	.48	.92	1.03	.27	.44	1.18
22, 10.15 a. m.							
22, 10.15 a. m.	.45	.48	1.09	.75	.25	.39	1.45
22, 12.10 p. m.							
Total	1.11	.96	2.01	1.78	.52	.80	1.31

TABLE I.—*Effect of Bordeaux mixture on the rates of transpiration of abscised leaves for periods before and after treatment*—Continued

PERIODS AFTER TREATMENT

Plant and period.	Transpiration quantities.						Ratio A′ : B.
	Series A′.			Series B (control).			
	Leaf 1.	Leaf 2.	Leaf 3.	Leaf 1.	Leaf 2.	Leaf 3.	
Ricinus communis:	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
Feb. 4, 9.30 a. m.	3.50	5.50	4.70	3.00	2.30	4.10	1.45
4, 3.30 p. m.							
5, 8.30 a. m.	3.60	3.40	4.40	3.10	2.80	4.00	1.15
5, 4.30 p. m.							
6, 12.00 a. m.	2.30	1.30	2.40	1.90	1.70	2.30	1.01
6, 5.30 p. m.							
Total	9.40	10.20	11.50	8.00	6.80	10.40	1.33
Datura metaloides:							
Mar. 18, 4.00 p. m.	5.00	7.10	3.00	3.10	1.30	2.70	2.13
19, 9.00 a. m.							
19, 9.00 a. m.	3.30	4.90	5.00	1.30	1.30	6.00	1.53
20, 4.00 p. m.							
Total	8.30	12.00	8.00	4.40	2.60	8.70	1.82
Phaseolus vulgaris:							
Mar. 22, 3.00 p. m.	.13	.24	.19	.11	.06	.14	1.80
22, 4.50 p. m.							
22, 4.50 p. m.	.09	.16	.12	.09	.10	.08	1.37
22, 6.50 p. m.							
Total	.22	.40	.31	.20	.16	.22	1.60
Beta cycla:							
Mar. 22, 3.55 p. m.	.19	.19	.22	.19	.32	.15	1.07
22, 5.55 p. m.							
22, 5.55 p. m.	.11	.17	.16	.12	.15	.14	1.07
22, 7.55 p. m.							
Total	.30	.36	.38	.31	.37	.29	1.07
Raphanus sativus:							
Mar. 22, 3.40 p. m.	.32	.52	.....	.19	.23	.21	1.33
22, 5.35 p. m.							
22, 5.25 p. m.	.21	.38	.....	.13	.30	.26	1.00
22, 7.35 p. m.							
Total	.53	.90	.....	.32	.43	.47	1.17
Caladium sp. (small variety):							
Mar. 22, 3.30 p. m.	.34	.10	.34	.06	.15	.02	3.39
22, 5.20 p. m.							
22, 5.20 p. m.	.09	.14	.16	.10	.13	.10	1.18
22, 7.25 p. m.							
Total	.43	.24	.50	.16	.28	.12	2.08
Hibiscus cardinalis:							
Mar. 22, 3.45 p. m.	.14	.14	.14	.05	.07	.04	2.62
22, 5.45 p. m.							
22, 5.45 p. m.	.09	.12	.18	.06	.06	.04	2.42
22, 7.40 p. m.							
Total	.23	.26	.32	.11	.13	.08	2.53
Clerodendron balfouri:							
Mar. 22, 3.10 p. m.	.08	.12	.12	.10	.09	.07	1.23
22, 5.05 p. m.							
22, 5.05 p. m.	.09	.10	.10	.08	.05	.04	1.70
22, 7.00 p. m.							
Total	.17	.22	.22	.18	.14	.11	1.46
Datura metaloides:							
Mar. 22, 3.15 p. m.	.72	.81	.18	.53	.28	.35	1.47
22, 5.15 p. m.							
22, 5.15 p. m.	.52	.59	.79	.37	.25	.22	2.26
22, 7.10 p. m.							
Total	1.24	1.40	.97	.90	.53	.57	1.80

A study of the last column of Table I brings out the fact that the first 2-hour period after spraying yielded higher ratio values than the second period after spraying. A notable exception to this, however, is the experiment with *Datura metaloides* conducted on March 22. Here the second period after spraying yields a ratio value considerably higher than the first, while in the experiment with Swiss chard the ratios for the two periods after spraying have the same value. The experiments with Datura sp. and with castor-bean leaves, extending over time periods of four days and five days, respectively, show a decrease in the ratio values for the time periods of successive days. It appears, therefore, that in these experiments the highest rate of water loss from the leaves due solely to the influence of the spray occurred soon after the spray had dried upon the leaves, certainly within the first 2-hour period, after which there was a gradual decrease in the transpiration rates as influenced by the surface film of the spray. As is clearly brought out in the experiment with castor-bean leaves, this gradual decrease in the rates of water loss from the leaves, as indicated by the ratio values, extends through a period of three days after spraying. The gradual decrease in the ratio values during the period after spraying is undoubtedly caused by the gradual disintegration and wearing away of the surface films. It may therefore be assumed that whatever may be the physical basis for the increased evaporation rates from plant surfaces following the application of Bordeaux mixture, a modification of leaf structure in response to a chemical stimulus induced by the spray can not be responsible for the increased transpiration rates.

In Table II is given a brief summary of the average data presented in Table I. The ratio values of total transpiration quantities for standardization periods are here considered as 1.00, while the corresponding ratio values for periods after spraying are expressed in terms of these.

TABLE II.—*Summary of the average data from Table I. Ratio values of A′ to B for periods after spraying are relative to the ratio values of A to B for standardization periods expressed as 1.00*

Plant.	Duration of experiment.	Transpiration (ratio A′:B).
Ricinus communis	6 days	1.50
Datura metaloides	4 days	2.12
Phaseolus vulgaris	10 hours	2.39
Beta cycla	do	1.57
Raphanus sativus	do	1.63
Caladium sp	do	1.81
Hibiscus cardinalis	do	3.72
Clerodendron balfouri	do	1.43
Datura metaloides	11 hours	1.37
Average		1.99

From the brief summary in Table II it will be observed that the lowest ratio value for any period after treatment is 1.37 times the ratio value for the corresponding standardization period, while the highest ratio value is 3.72 times the ratio value for the corresponding standardization period. The average value of the ratios for periods after spraying is 1.99 times the average ratio for the standardization periods. Expressed in another way, the leaves sprayed with Bordeaux mixture showed an increase in the rates of water loss, relative to their respective controls in each case, varying from 37 per cent for the lowest increase to 272 per cent for the highest. The average rate of water loss by transpiration of the species here employed shows an increase of 99 per cent over the average rate for the standardization period. The degree of this accelerating influence of Bordeaux mixture on the rates of transpiration varies considerably with the different species of leaves, as is indicated by this wide range of variation in the ratio values for the different leaves.

The leaves employed in these experiments were allowed to remain in the places occupied during the experiments for some time after the experiments had been terminated and were kept under observation to determine whether the sprayed or the unsprayed leaves should first show signs of wilting. It was observed that in every instance the sprayed leaves showed signs of wilting at some period preceding the time at which the unsprayed leaves of the same species began to wilt. This is only what would be expected in view of the fact that the average rate of water loss from the sprayed leaves is nearly double that from the unsprayed leaves during the experimental time period.

EXPERIMENTS WITH POTTED PLANTS

EXPERIMENTAL METHODS.—In the experiments with potted plants the method of procedure was similar to that followed in the experiments with abscised leaves. The plants employed consisted of tomato (*Lycopersicon esculentum* Mill.), cabbage (*Brassica oleracea* L.), pepper (*Capsicum annuum* L.), egg plant (*Solanum melongena* L.), and soy bean (*Glycine hispida* M.). These plants; excepting the soy beans, were grown in beds of soil in the greenhouse until they had attained a size suitable for experimentation of this character. They were then transplanted to earthenware pots glazed inside and outside and having a capacity of approximately 1.5 liters. In order to prevent evaporation from the surface of the soil, melted wax prepared according to the Briggs and Shantz formula was poured over the surface of the soil in each pot, thus making a perfect seal.

Water was automatically supplied to the roots of the plants by means of the autoirrigator. This instrument has been described by Livingston (12) and later by Hawkins (11). It consists of a porous clay cup closed by a rubber stopper through which extend two glass tubes. One of these tubes is bent into the form of an inverted U having one arm considerably

longer than the other. The short arm extends through the rubber stopper into the cup. The bend of the U turns over the edge of the container and the end of the longer arm dips into a reservoir of water below. The second glass tube passes through the rubber stopper and ends just at its lower surface. To the upper end of this glass tube a short piece of rubber tubing is attached. This is closed by means of a pinchcock or screw clamp when the instrument is installed. In order to install the instrument, the rubber stopper with its glass tubes is pressed firmly into the mouth of the cup. The cup is then buried in the soil of the container among the roots of the plant. The end of the long arm of the U-tube is dipped into a suitable reservoir of water below. Suction is now applied to the short rubber tube. This causes water to rise through the U-tube into the cup, filling the latter. When the water has risen and filled the cup and the short rubber tube, a pinchcock is applied, thus closing the tube. In installing the instrument, care must be taken that all connections are tight and that all air is removed from the system. As water is removed from the soil by the plant roots, more water passes through the porous walls of the clay cup, replacing that absorbed by the roots. In this way an approximately constant soil-moisture content may be maintained. Any desired moisture content of the soil may be obtained and maintained approximately by simply increasing or decreasing the distance between the surface of the water in the reservoir and the surface of the water in the porous clay cup.

In experiments such as are here reported, a constant moisture content of the soil in which the plants are rooted is of considerable importance, and the old method of supplying water to the roots at stated intervals has proved unsatisfactory.

After installing the autoirrigators and sealing the pots the plants were allowed to stand a week in order to become adjusted to the new conditions before the experiments were begun.

In each experiment 12 plants were employed. These were chosen from a much larger number and were selected with special reference to uniformity of size and vigor. The 12 plants were divided into two groups, each group constituting a series of 6 plants. In order to expose the plants included in a single experiment to the constantly changing aerial conditions in such a way that all might be affected in a somewhat similar manner, they were arranged in two rows, 6 plants to a row, on opposite sides of a greenhouse bench, the rows being placed near the edge of the bench. The bench was centrally located and was not in the direct path of air currents from open doors or ventilators. As a further precaution, the plants were shifted in their positions in the rows each day according to a definite plan previously decided upon.

The plants were weighed each day and the water loss from each plant for the time period immediately preceding was determined. The weighings were made in the order in which the plants were numbered, from 1

to 6, and this plan was maintained throughout the experiment, so that the time periods for each plant between any two weighings were approximately the same. At the end of the standardization period the plants of series A were sprayed. This series now became series A′. The Bordeaux mixture employed was prepared according to the same formula as that used in the experiments with abscised leaves. The spray was applied to the leaves of the plants with an atomizer. This method gives a very uniform film of the spray over the leaf surfaces. The spray was applied to both the upper and lower surfaces of the leaves, except in the experiments with cabbage plants.

At the close of an experiment the tops were severed from the roots just at the surface of the wax seal, and the green weights were immediately obtained. The plants were then placed in weighing bottles and dried in an oven at a temperature of from 76° to 100° C. for a period of 28 hours, after which they were dried to constant weight at a temperature of from 102° to 105° C. The bottles were then transferred to a large desiccator and were allowed to cool to room temperature before weighing.

Measurements were taken of the evaporating power of the air in the greenhouse room where the experiments were carried out. These measurements were made by means of standardized porous-cup atmometers (13); the instruments were placed among the plants on the greenhouse bench and readings were taken each day at the time when the plants were weighed. The readings were corrected to the Livingston cylindrical standard by multiplying by the coefficient of correction of the cup used. In the time during which the experiments were conducted the water loss from the porous-cup atmometer gave a daily mean of 10.4 c. c.; a maximum daily rate of 24.0 c. c. (on March 1) and a minimum daily rate of 6.0 c. c. (on March 6).

As in the experiments with abscised leaves, one of the two groups of plants comprised in a single experiment will be designated "series A." The other group, remaining untreated throughout the experiment, will be designated "series B." In the experiments with potted plants transpiration quantities for series A and for series B will be denoted "A" and "B" in their respective series. This method of notation is precisely the same as that adopted in the experiments with abscised leaves.

In presenting the data for the experiments with potted plants the ratios between the water loss per gram of green substance for periods before and after spraying, as well as the corresponding water loss per gram of dry substance, will also be treated. Green- and dry-weight values could, of course, not be obtained until the close of an experiment. These values must serve, therefore, for the calculation of ratios for the standardization periods as well as for the periods after spraying. This, however, could make no material difference in the ratio values for periods before spraying, since any increase in the weight of plant substance due

to the growth of the plants during the periods after spraying would affect approximately alike the green- and dry-weight values of both series A′ and series B. If, in the treatment of these ratios the green-weight values for series A and for series A′ (the values for these two series being the same) are denoted by "G," and the corresponding values for series B are denoted by "G′," it follows that the ratios of A to G, A′ to G, and B to G′ must represent the water loss per gram of green substance for series A, series A′, and series B, respectively. Now, the ratio between the average water loss per gram of green substance for series A and for series B (periods before spraying) is expressed by $\frac{A:G}{B:G'}$, or $\frac{AG'}{BG}$. The corresponding ratio value between the average water loss per gram of green substance for series A′ and for series B (periods after spraying) is expressed by $\frac{A':G}{B:G'}$, or $\frac{A'G'}{BG}$. If, therefore, the ratio of AG′ to BG is less than that of A′G′ to BG, the water loss per gram of green substance must be greater for the sprayed plants than for the unsprayed.

The dry-weight values for series A′ and for series B will be denoted by "D" and "D′," respectively, and the ratios between the water loss per gram of dry substance for periods before spraying and the corresponding ratio for periods after spraying, derived in the same manner as the ratios between the water loss per gram of green substance, are expressed by the ratios of AD′ to BD and A′D′ to BD, for standardization periods and for periods after spraying, respectively.

CABBAGE PLANTS

In this experiment the plants used were vigorous and fairly uniform in size. The standardization period continued from March 1 to March 4. The period after spraying began on March 4 and continued to March 17.

Considerable difficulty was encountered in attempts to spray the leaves of the cabbage plants. The bloom on the surface of the leaves prevented the spray material from adhering. Various substances were added to the spray material in an attempt to find something which would cause the spray to adhere to the leaves. Rosin-fish-oil soap in the proportion of 2 pounds to 50 gallons of Bordeaux mixture, as recommended by Hawkins (10), proved to be the most effective; but even this did not give the desired results. It was found that by lightly brushing the surface of the leaves with a wad of dry absorbent cotton, and by immediately afterwards applying the spray, a surface film of the spray could be obtained. This film was by no means ideal, but it was better than that secured by using the rosin-fish-oil soap in connection with the Bordeaux mixture. The leaves of the control series B were also lightly brushed with a wad of absorbent cotton at the same time that the leaves of series A′ were thus treated. Only the upper surfaces of the leaves were thus treated and sprayed.

In Table III are given the data for the experiment with potted cabbage plants. This table presents first in a horizontal section the summed transpiration quantities for each plant and the average for the plants in each series. It also gives the green and dry weight values for each plant and the average for the plants in series A′ and in series B. This section is followed below and in the order here given by (1) the ratios between the average transpiration quantities of the two series for the periods before and after spraying; (2) the ratios between the quantities representing the average water loss per gram of green substance, of the two series in periods before and after spraying; and (3) the ratio between quantities representing the average water loss per gram of dry substance, of the two series in the periods before and after spraying. The difference between the two ratios in a pair, on the same horizontal line, is given to the right. The plus (+) sign following the difference indicates that the ratio for the period after spraying is higher than the corresponding ratio for the standardization period.

TABLE III.—*Effect of Bordeaux mixture on the rate of transpiration of potted cabbage plants*

[Period before treatment, Mar. 1 to Mar. 3; period after treatment, Mar. 4 to Mar. 17, 1916]

Plant No.	Transpiration quantities.				Green weight of tops.		Dry weight of tops.	
	Period before treatment.		Period after treatment.					
	Series A.	Series B.	Series A′.	Series B.	Series A′.	Series B.	Series A′.	Series B.
	(*A*)	(*B*)	(*A′*)	(*B*)	(*G*)	(*G′*)	(*D*)	(*D′*)
1	67.6	33.4	1015.2	342.1	56.48	36.92	10.48	6.15
2	46.5	32.7	709.0	523.9	58.28	39.18	8.98	7.70
3	71.1	36.1	1041.9	493.3	58.81	40.72	10.90	6.20
4	42.6	30.7	642.4	585.5	42.82	38.11	7.74	6.99
5	60.5	33.4	1008.8	685.5	69.90	57.46	9.92	10.04
6	32.2	38.3	516.8	497.9	42.11	49.27	9.11	8.76
Average	53.4	34.1	835.7	521.4	54.72	43.61	9.36	7.64
Transpiration ratio	A: B=1.56		A′: B=1.60		Difference			0.04+
Water loss per gram of green weight ratio	AG′: BG=1.24		A′G′: BG=1.28		Difference			.04+
Water loss per gram of dry weight ratio	AD′: BD=1.27		A′D′: BD=1.31		Difference			.04+

It will be observed from Table III that the ratio values for the period after treatment are, in each case, slightly higher than the corresponding ratio values for the period before treatment. This indicates that the average rate of water loss by transpiration from the sprayed plants (series A′) was slightly higher, relative to the average rates from the control plants, than was the corresponding ratio of water loss from those same plants (series A) for the period before treatment. It is to be noted, however, that while the ratios between average quantities for the period after spraying are all higher than the corresponding ratios for the period before treatment, the individual ratios for the period after treatment

vary on either side of the average ratios for the standardization period. In this experiment with cabbage plants it may not, therefore, be stated with entire certainty that an application of Bordeaux mixture has an accelerating influence on the rates of water loss, since the difference between the ratio values for the periods before and after treatment are no greater than might be expected to lie within the limits of experimental error. On the other hand, the spray was applied only to the upper surfaces of the leaves, so that only one-half of the transpiring surface was affected by the spray material. Furthermore, stomatal transpiration from the upper surfaces of the leaves should be somewhat less than from the lower surfaces, considering the number of stomata per square millimeter on the upper leaf surface to be 219 and that on the lower surface to be 301, according to Duggar (5, p. 91). This would still further tend to minimize any accelerating influence which the spray might have on the rates of water loss.

PEPPER PLANTS

The plants used in the experiment with peppers were very uniform in size, about 8 inches tall, vigorous, and were just beginning to bloom when the experiment was begun. The experiment continued during a time period of 16 days. The standardization period extended from February 26 to March 3; the period after treatment continued from March 3 to March 14. The results of this experiment are given in Table IV. The arrangement of this table and subsequent ones is precisely the same as that employed in the presentation of the data for the experiment with cabbage plants.

TABLE IV.—*Effect of Bordeaux mixture on the rate of transpiration of potted pepper plants*

[Period before treatment, Feb. 26 to Mar. 3; period after treatment, Mar. 3 to Mar. 14, 1916]

Plant No.	Transpiration quantities.				Green weight of tops.		Dry weight of tops.	
	Period before treatment.		Period after treatment.					
	Series A.	Series B.	Series A'.	Series B.	Series A'.	Series B.	Series A'.	Series B.
	(*A*)	(*B*)	(*A'*)	(*B*)	(*G*)	(*G'*)	(*D*)	(*D'*)
1	38.1	35.7	109.3	43.0	3.70	5.02	0.75	0.83
2	37.9	17.0	91.4	37.7	2.08	2.57	.45	.31
3	31.4	27.9	63.3	42.5	4.57	2.25	.70	.40
4	37.3	40.0	102.8	103.3	4.40	4.87	.65	.73
5	29.8	32.9	65.2	54.7	4.20	4.73	.69	.78
6	57.9	20.9	114.8	37.9	6.80	2.75	1.12	.47
Average	38.7	29.0	91.1	53.1	4.29	3.69	.72	.58
Transpiration ratio	AB=1.33		A'B=1.71		Difference		0.38+	
Water loss per gram of green substance ratio	AG' BG=1.14		A'G' BG=1.47		Difference		.33+	
Water loss per gram of dry substance ratio	AD' BD=1.07		A'D'BD=1.37		Difference		.30+	

A comparison of the ratio values in Table IV shows very clearly the accelerating influence of a film of Bordeaux mixture on the rates of transpiration of pepper plants. If each of the three ratio values for the period before spraying is made equal to 1.00, the corresponding ratio values for the period after spraying are, in every case, 1.29—that is, 29 per cent higher than the values for the standardization period.

SOY-BEAN PLANTS

The soy-bean plants here employed were about 20 cm. tall when the experiment was begun. Soy-bean seedlings were transplanted directly into the containers when they were about 5 cm. tall. Three seedlings constituted a culture. Four series of cultures were employed, each series comprising six cultures and a total of 18 plants. The four series will be designated series A, series B, series C, and series D, respectively, for the standardization period. Series D is here considered the control series and remained untreated throughout the experiment. The plants of the first three series were treated at the end of the standardization period and are designated "series A′," "series B′," and "series C′," in the order given, for the period after treatment. As in all the experiments here reported, the leaves of the plants of series A′ were covered with a film of Bordeaux mixture. The leaves of series B′ were sprayed with a suspension of barium sulphate in water. The mixture consisted of 28 gm. of barium sulphate in 1 liter of water. This was applied by means of an atomizer in precisely the same manner in which the Bordeaux mixture was applied. The leaves of series C′ were treated with dry copper sulphate in the form of a fine powder. The copper sulphate was prepared by gently heating the crystals in a porcelain crucible until all the water of crystallization had been driven off. After the salt had thus been dried to constant weight, it was ground to a fine powder in a mortar and kept in a desiccator until used. This powder was dusted on the upper surfaces of the leaves until a thin but fairly uniform covering was obtained. During the time period of the experiment the copper sulphate produced no injurious effects upon the leaves, though during this period care was taken to keep the greenhouse room as dry as possible.

For the sake of convenience in presenting the data for this experiment, the transpiration quantities for series A, series B, series C, and series D are denoted by "A," "B," "C," and "D," respectively, for the standardization period; the transpiration quantities for the corresponding series for the period after spraying are denoted by "A′," "B′," "C′," and "D," respectively.

The period of standardization extended from February 29 to March 4; the period after treatment continued from March 4 to March 11. The data for this experiment are presented in Table V.

TABLE V.—*Effect of Bordeaux mixture, copper sulphate, and barium sulphate on the rate of transpiration of potted soy-bean plants*

[Period before treatment, Feb. 29 to Mar. 4: period after treatment, Mar. 4 to Mar. 11, 1916]

Plant No.	Transpiration quantities.							
	Period before treatment.				Period after treatment.			
	Series A (Bordeaux mixture).	Series B (barium sulphate).	Series C (copper sulphate).	Series D (control).	Series A′ (Bordeaux mixture).	Series B′ (barium sulphate).	Series C′ (copper sulphate).	Series D (control).
	(*A*)	(*B*)	(*C*)	(*D*)	(*A′*)	(*B′*)	(*C′*)	(*D*)
1	34.2	53.0	49.0	53.1	128.1	131.3	195.6	177.9
2	64.0	34.0	56.6	58.2	254.7	77.4	198.6	130.2
3	77.5	45.8	39.4	33.5	304.8	103.5	124.9	76.4
4	65.8	38.7	33.5	65.3	217.8	88.7	75.6	142.3
5	67.9	46.5	82.0	46.6	265.1	135.6	252.0	94.8
6	55.6	37.5	92.3	43.9	227.8	87.7	253.2	85.7
Average	60.8	42.5	58.8	50.1	233.0	104.0	183.3	117.8
Transpiration ratio	A D= 1.21	B D= 0.85	C D= 1.17	.....	A′ D= 1.98	B′ D= 0.88	C′ D= 1.55	.....
Difference	.....	.....	.....	.....	0.77+	0.03+	0.38+	.....

From the data of Table V it appears that of the three kinds of materials applied to the leaves of soy-bean plants Bordeaux mixture is the most effective in bringing about an increased rate of water loss. The plants sprayed with Bordeaux mixture here showed, relative to the control plants, an average rate of water loss 63 per cent higher than the average rates of the same plants before spraying. The leaves of the plants dusted with copper sulphate showed a corresponding increase of 37 per cent, while the plants sprayed with barium sulphate show an increase in the average rate of water loss, relative to the control plants, of only 3 per cent over the average rate for the standardization period.

The results here obtained are in entire accord with the conclusions reached by Duggar and Cooley (6) that certain specific characters of the films are important factors, considered in relation to the modified rates of transpiration. The further suggestion of these authors that modified leaf temperature induced by heat absorption due to the color of the surface films is an important factor might also be considered as effective in bringing about the results obtained here, since a film of Bordeaux mixture, being darker in color than either of the two other surface coverings used may be assumed, without any other direct evidence, to have a higher heat-absorbing power than either copper sulphate or barium sulphate. It is scarcely probable, however, that modified leaf temperatures, induced by differences in the heat-absorbing powers of the surface films here used, could account for any great portion of the differences in the rates of water loss observed.

EGGPLANTS

In the experiment with eggplants the standardization period extended two days from April 3 to April 5. The period after spraying continued from April 5 to April 18.

The results of this experiment are presented in Table VI.

TABLE VI.—*Effect of Bordeaux mixture on the rate of transpiration of potted eggplants*

[Period before treatment, Apr. 3 to Apr. 5; period after treatment, Apr. 5 to Apr. 18, 1916]

Plant No.	Transpiration quantities.				Green weight of tops.		Dry weight of tops.	
	Period before treatment.		Period after treatment.					
	Series A.	Series B.	Series A′.	Series B.	Series A′.	Series B.	Series A′.	Series B.
	(*A*)	(*B*)	(*A′*)	(*B*)	(*G*)	(*G′*)	(*D*)	(*D′*)
1	20.9	17.4	400.1	326.2	12.02	11.73	1.67	1.68
2	27.9	23.6	336.2	316.6	11.12	12.63	1.87	1.83
3	23.4	40.0	295.9	426.2	11.35	18.10	1.58	2.72
4	27.4	26.5	445.7	367.0	15.11	14.62	2.14	1.77
5	30.1	33.7	494.1	394.2	19.62	14.19	1.97	1.99
6	18.0	15.9	292.1	334.4	12.40	11.60	1.52	1.55
Average	24.6	26.1	377.3	360.7	13.60	13.81	1.79	1.92
Transpiration.....ratio..	A : B=0.94		A′ : B=1.04		Difference.....			0.10+
Water loss per gram of green substance.....ratio..	AG′ : BG=0.95		A′G′ : BG=1.06		Difference.....			.11+
Water loss per gram of dry substance.....ratio..	AD′ : BD=1.00		A′D′ : BD=1.12		Difference.....			.12+

The results given in Table VI show a pronounced increase in the rate of water loss for the sprayed plants relative to the rates from the control plants, as is indicated by the higher ratio values for the period after spraying. The value of the ratio between transpiration quantities for the period after treatment is here 11 per cent higher than the corresponding ratio value for the period before treatment. Ratios between quantities representing water loss per gram of green weight and water loss per gram of dry weight are each 12 per cent higher for the period after spraying than for the standardization period.

TOMATO PLANTS

It has been previously stated that the Bordeaux mixture employed throughout these experiments was prepared according to the 5–5–50 formula of agricultural practice. When this mixture was applied to tomato plants, however, injury to the leaves resulted. In this experiment, therefore, the strength of the mixture was reduced to conform approximately to the 4–4–50 formula commonly used. This mixture produced no injurious effects upon the plants here employed.

In this experiment the period of standardization extended from May 26 to June 1; the period after treatment continued from June 1 to June 12. The experimental data are presented in Table VII.

TABLE VII.—*Effect of Bordeaux mixture on the rate of transpiration of potted tomato plants*

[Period before treatment, May 26 to June 1; period after treatment, June 1 to June 12, 1916]

Plant No.	Transpiration quantities.				Green weight of tops.		Dry weight of tops.	
	Period before treatment.		Period after treatment.					
	Series A.	Series B.	Series A'.	Series B.	Series A'.	Series B.	Series A'.	Series B'.
	(*A*)	(*B*)	('*A*)	(*B*)	(*G*)	(*G'*)	(*D*)	(*D'*)
1	231.2	210.6	245.0	231.9	17.70	16.76	2.40	2.23
2	260.8	182.2	269.9	268.9	17.50	14.65	2.30	1.77
3	143.0	170.6	238.3	203.2	19.15	14.72	2.01	1.87
4	171.7	172.5	226.5	207.0	15.12	15.05	2.02	2.00
5	215.8	171.0	291.2	142.4	21.03	15.52	2.68	1.98
6	184.5	212.0	202.5	214.1	13.95	14.01	1.92	1.92
Average	201.1	186.4	245.5	211.2	17.40	15.11	2.20	1.94
Transpiration ratio	AB=1.07		A'B=1.16		Difference			0.09+
Water loss per gram of green substance ratio	AG' BG=0.93		A'G' BG=1.01		Difference			.08+
Water loss per gram of dry substance ratio	AD' BD=0.95		A'D' BD=1.02		Difference			.07+

From Table VII it will be observed that the ratios between transpiration qualities and between quantities representing water loss per gram of green substance for the periods after spraying are 8 per cent and 9 per cent higher, respectively, than the corresponding ratio values for the periods before spraying, while the ratio between quantities representing water loss per gram of dry substance is 7 per cent higher for the period after treatment. The average transpiration rate for the sprayed plants, relative to the control plants, is therefore 8 per cent higher than the average relative rate for the same plants before treating them with Bordeaux mixture.

Table VIII presents a brief summary of the average data contained in Tables III, IV, V, VI, and VII. The values of the ratios between average transpiration quantities, between average water loss per gram of green substance and between average water loss per gram of dry substance, for the periods after spraying, are here given in terms of the corresponding ratio values for the standardization periods considered as unity. The ratio values for the standardization periods are therefore omitted from the table, since all have the same relative value (1.00).

A comparison of the ratio values presented in this brief summary shows that the treated plants of each species here dealt with gave higher average rates of transpiration, relative to the rates from their respective controls, than did the same plants during the standardization periods. The lowest average rates of water loss for periods after treatment occurred with cabbage plants, showing an average increased rate of 2 per cent over the corresponding rate for the standardization period. It has already been pointed out that the low increased rates of water loss

recorded for cabbage plants may be due to the fact that the leaves were sprayed only on the upper surfaces, and that at best only a very imperfect film was obtained.

TABLE VIII.—*Average ratio values for periods after treatment, relative to the corresponding ratio values for the standardization periods taken as unity, being a summary of the average data from Tables III, IV, V, VI, and VII*

Plant.	Transpiration (ratio A′:B).	Water loss per gram of dry substance (ratio A′G′:BG).	Water loss per gram of green substance (ratio A′D′:BD).
Cabbage	1.03	1.03	1.03
Eggplant	1.11	1.12	1.12
Pepper	1.29	1.29	1.29
Tomato	1.08	1.09	1.07
Soy bean	1.64	.....	.....

It is further to be noted that with each species employed in these experiments the three ratios, each derived from a single set of the three kinds of measurements here dealt with—transpiration, water loss per gram of green substance, and water loss per gram of dry substance—are in very close agreement. The greatest variation in the values of the three ratios occurred with tomato plants. The values of the ratios in question are 1.08, 1.09, and 1.07 for transpiration quantities, water loss per gram of green substance, and water loss per gram of dry substance, respectively. The influence of Bordeaux mixture in bringing about increased rates of transpiration varies with the different species of plants, as is indicated by the variation in the ratio values for the different species. This has already been observed in the experiments with abscised leaves.

A comparison of the results obtained from abscised leaves with those obtained from potted plants shows very clearly that the influence of Bordeaux mixture in bringing about increased rates of transpiration is much more pronounced when the spray is applied to abscised leaves than when applied to the leaves of potted plants. Thus, the ratio values representing the highest and lowest average increased rates of water loss from abscised leaves, due to an application of Bordeaux mixture, are 3.71 and 1.73, respectively, relative to the corresponding ratio values for the standardization periods taken as unity. The ratio values representing the highest and lowest average increased rates of transpiration from potted plants, following an application of Bordeaux mixture, are 1.64 and 1.02, respectively, relative to the corresponding ratio values for the time period before spraying.

SUMMARY

The results of the experiments presented above substantiate the general principle already established by Duggar and Cooley (6, 7) that the rates of transpiration from abscised leaves and also from the leaves

of potted plants are materially increased by an application of Bordeaux mixture.

A surface covering of dry, powdered copper sulphate was less effective in accelerating rates of transpiration than was a surface film of Bordeaux mixture, but was more effective than was a film of barium sulphate.

The accelerating influence of Bordeaux mixture on the rates of transpiration is much more pronounced when the spray is applied to abscised leaves than when applied to the leaves of potted plants.

The influence of Bordeaux mixture in increasing the rates of water loss from abscised leaves becomes effective immediately after the spray dries upon the leaves. The highest average increased rates of transpiration occurred during the first 2-hour period following an application of the spray.

The effectiveness of a film of Bordeaux mixture for inducing increased rates of water loss from abscised leaves varies considerably with the different species. This, to a lesser degree, is true also with potted plants.

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